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A CYTOLOGICAL STUDY OF CARTERIA CRUCIFERA[1]

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(WITH THIRTY-NINE FIGURES)

The genus *Carteria* was named by Diesing (1866), whose type species was *C. cordiformis*, previously described by Carter (1858) as *Cryptoglena cordiformis*. The original description of the species used in this study, *Carteria crucifera* Korshikov, is apparently that given by Pascher (1927), since no description by Korshikov has been found prior to that included in his "Volvocineae" (1938). It is probable that Pascher obtained the description from Korshikov by private communication.

Kofoid and his students have shown that centrosomes and a neuromotor apparatus occur in many animal-like flagellates. Reports of similar structures in the plant-like flagellates have been viewed with skepticism by phycologists because of the incomplete and contradictory evidence. *C. crucifera* was selected for study for two reasons. First, it had been suggested that there might be a correlation between the double number of flagella in *Carteria* and the number of chromosomes. Since the chromosome number had been reported for several species of *Chlamydomonas*, there was a basis for an approximate comparison. Secondly, the position of the pyrenoid anterior to the nucleus reduces the danger of mistaking the narrow layer of cytoplasm between the contractile vacuoles for a rhizoplast.

MATERIALS AND METHODS

The strain of *Carteria crucifera* used for this study was isolated in 1927 by Dr. R. I. Evans from a sample collected in Durham, N. C., and has since

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been kept in culture on nutrient agar[2] at the University of Wisconsin. Stock cultures grew most satisfactorily when kept at about 18° C. under constant illumination. Rapidly growing motile cells were obtained by transferring portions of the agar cultures to low bulb pots filled with quartz sand which were placed in pot-saucers containing a solution composed of about 300 cc. of distilled water to 15 cc. each of Knop's solutions A and B. The cultures were almost entirely covered with glass plates and kept in the greenhouse at 60–65° F.

Nuclear division figures were most abundant in cultures which had been growing on sand for 5–10 days. Counts made at 7 a.m. and 7 p.m. for several periods of three weeks each showed no appreciably greater increase in the number of cells during the night than during daytime. Evidently under cultural conditions cell division is not limited to periods of darkness.

Material to be fixed was centrifuged, mixed with fresh egg albumen, and then smeared on slides and cover glasses which were dropped face down into the fixative. Some of the centrifuged material was placed in vials, fixed, and imbedded in paraffin. To prevent loss of the material, the paraffin was allowed to harden in the narrow vials, and after it was cold the vials were broken. Sections were cut 2–3 μ thick. Schaudinn's alcohol-sublimate solution, with or without acetic acid, used either hot or cold, was the most satisfactory fixative. Carnoy's alcohol-acetic solution, Flemming's medium solution and Randolph's and Belling's modifications of Navashin's fixative also gave satisfactory results.

Some of the preparations were stained with Heidenhain's iron-alum haematoxylin and counterstained with 0.1 per cent Bordeaux red. This method brought out the nucleus, pyrenoid, and the structure of the cytoplasm. Feulgen's nucleal reaction (Robertson 1927) counterstained with fast green proved the most satisfactory method for demonstrating nuclear structure, since neither the nucleolus nor any cytoplasmic structures were stained. Ehrlich's haematoxylin was satisfactory for both nuclear and cytoplasmic structures. Flemming's triple stain was used to study the chloroplast in sectioned material, and F. H. Smith's (1934) modification of the crystal violet-iodine stain was helpful in observing starch formation. Living cells were treated with an aqueous solution of iodine (0.15 per cent) in potassium iodide (0.3 per cent) to identify starch.

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[2] To one liter of 1.4% plain agar the following salts were added:

Potassium nitrate	1.0 gram
Calcium sulphate	0.5 "
Magnesium sulphate	0.5 "
Ferrous phosphate	0.25 "
Tribasic calcium phosphate	0.25 "

Eight-ounce screw-cap bottles, each containing about 60 cc. of the agar, were sterilized and slanted.

OBSERVATIONS

The living motile cells of *C. crucifera* are ellipsoidal and slightly asymmetrical, one side often appearing more convex than the other in optical section (fig. 1). The cells range from 15 to 22 μ in length and from 7 to 14 μ in width. The size varies with cultural conditions and with different stages of growth. The wall (pellicle) is distinct and is thickened anteriorly to form a relatively large papilla. In a surface view looking toward the anterior end of the cell this protuberance presents somewhat the shape of a cross, and the four flagella project from the cell through fine canals, one in each of the depressions between the arms of the cross. The light green chloroplast appears to fill the cell except for a small concave region in the anterior end. Occasionally there is a small irregular clear region at the posterior end. In surface view longitudinal striations of the chloroplast are visible. A small ovoid red pigment-spot is located either in the outer layer of the chloroplast or between the wall and the chloroplast in the anterior half of the cell (fig. 1).

The pyrenoid occupies the greater portion of the middle part of the cell and is surrounded by a small number of angular starch grains which form an interrupted shell about it. Two contractile vacuoles, which contract alternately, are located in the anterior end of the cell. The contraction is rapid, but the interval between contractions is long as compared with that in ciliates. In a living cell the nucleus can be identified only as a small, spherical, nearly colorless region in the posterior part of the cell.

The thickness of the cell and the abundance of starch grains make it difficult to determine the shape of the chloroplast in living cells. In fixed and stained material it was not possible to distinguish between chloroplast and undifferentiated cytoplasm, and in the following discussion both will be included under the term "cytoplasm." The cytoplasm generally appears alveolar; in an occasional cell, however, it seems to be granular. In cross section a variable number of strands are visible extending from the peripheral cytoplasmic later to the pyrenoid, which is located centrally (fig. 4) or more or less laterally (fig. 5). In a cross section anterior to the pyrenoid, strands extend from the periphery and meet in the center forming a meshwork (fig. 6). It was not possible to determine whether the strands which extend from the peripheral portion of the cytoplasm to the pyrenoid belong to the chloroplast or to the undifferentiated cytoplasm. If to the latter, this would be one of the few species in which the pyrenoid is located outside the chloroplast, a condition reported by Kater (1929) for *Chlamydomonas*. It is probable, however, that *C. crucifera* possesses what is commonly described as an "H-shaped" chloroplast (really H-shaped in longitudinal section), the pyrenoid being imbedded in the strands running from the peripheral portion.

The longitudinal striations of the chloroplast are visible in whole mounts

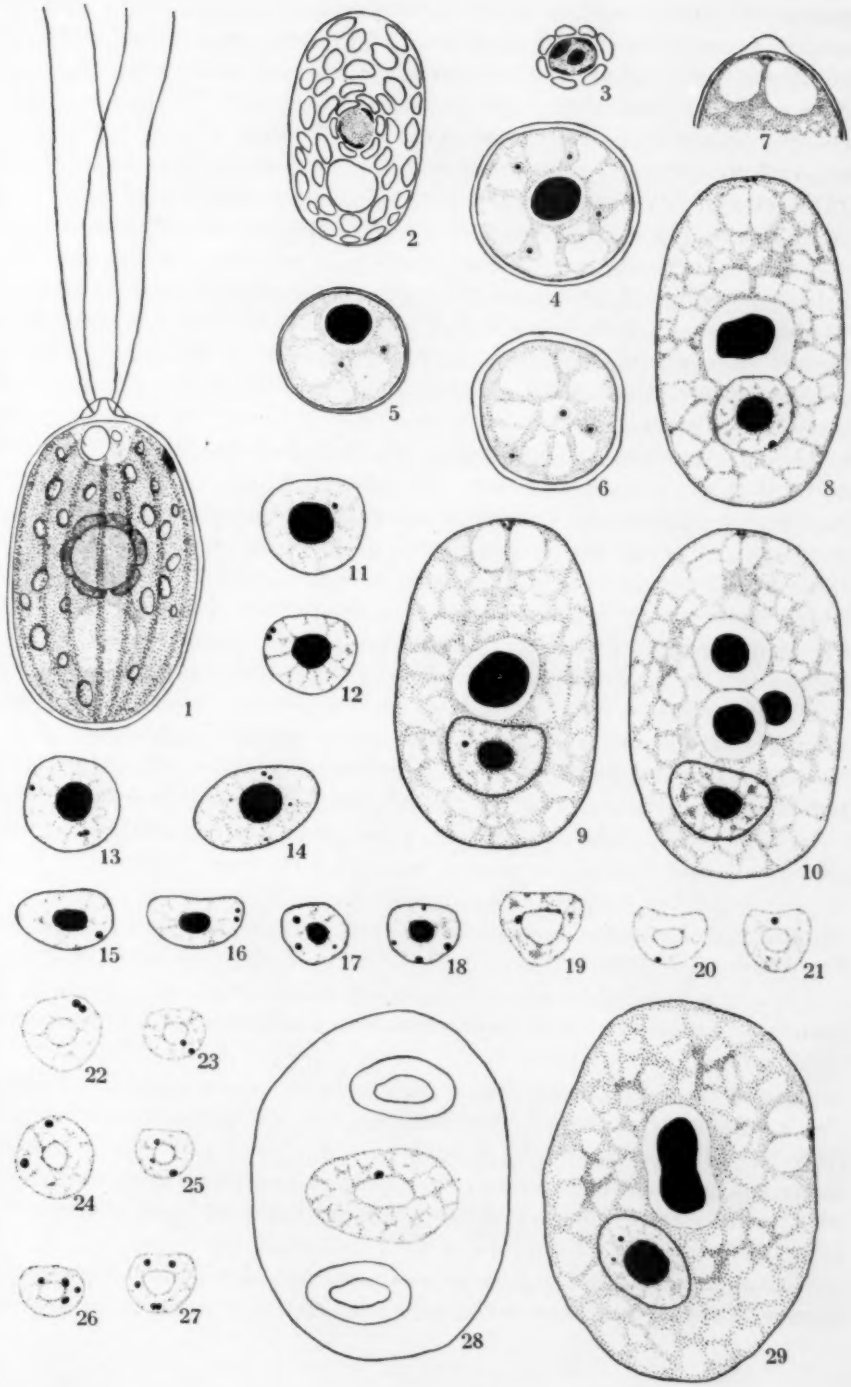
stained with Heidenhain's iron-alum haematoxylin or with Ehrlich's haematoxylin. When a living cell is seen from the anterior or posterior end there is some indication of ridges. In cross sections of fixed and stained material there is no such indication (figs. 4, 5, 6). Similar appearances in *Chlamydomonas* led Kater (1929) to suggest that the striated effect is produced as much by differences in opacity within the chloroplast as by the presence of actual ridges. It is possible that the difficulty in distinguishing between chloroplast and undifferentiated cytoplasm explains the apparent absence of ridges in cross sections of fixed material.

In a living cell the spherical pyrenoid is surrounded by a small number of concavo-convex bodies which give a starch reaction with iodine. After fixation with Schaudinn's fluid the pyrenoid shows a marked affinity for the Heidenhain stain, which colors it homogeneously black (figs. 8, 9). If preparations are stained with Bordeaux red before they are placed in haematoxylin, the pyrenoid becomes bright red. With Ehrlich's haematoxylin the pyrenoid is a light reddish purple. In either haematoxylin stain the pyrenoid is surrounded by a clear zone of varying thickness. This zone is probably occupied partially by the unstained shell of starch grains already mentioned. There is no indication of a reticulate structure of the pyrenoid or of radial strands traversing the hyaline zone about the pyrenoid, both of which were reported by Kater (1929) in *Chlamydomonas*. After Flemming's triple stain the bright red pyrenoid is surrounded by a layer of blue starch grains. The pyrenoid is stained light blue by crystal violet–iodine, while the surrounding starch grains are purple. Between the pyrenoid and the starch grains is a narrow hyaline zone (fig. 2). Several regions of the outer portion of the pyrenoid are usually stained dark blue (fig. 2). When these dark-staining

Explanation of figures 1–29

All drawings were made with a camera lucida; ×2550. Unless otherwise stated, all figures are from material fixed in Schaudinn's fluid.

FIG. 1. *Carteria crucifera* drawn from life. FIGS. 2, 3. Cells fixed in Randolph's modification of Navashin's fixative and stained with crystal violet-iodine, showing the pyrenoid with its outer transitional portion and the starch grains. FIGS. 4–10. Cells stained with Heidenhain's iron-alum haematoxylin. FIG. 4. Cross section showing centrally located pyrenoid. FIG. 5. Cross section with laterally located pyrenoid. FIG. 6. Cross section above level of pyrenoid. FIG. 7. Longitudinal section showing two blepharoplasts, contractile vacuoles and layer of cytoplasm between vacuoles. FIG. 8. Vegetative cell with two blepharoplasts, layer of cytoplasm between vacuoles, pyrenoid and nucleus. FIG. 9. Vegetative cell showing three blepharoplasts, layer of cytoplasm between contractile vacuoles, nucleus and pyrenoid. FIG. 10. Vegetative cell with two blepharoplasts, three pyrenoids and nucleus. FIGS. 11–14. Nuclei stained with Heidenhain's iron-alum haematoxylin. FIGS. 15–18. Nuclei stained with Ehrlich's haematoxylin showing number and arrangement of chromatic bodies. FIGS. 19–28. Nuclei stained with Feulgen's nucleal reaction. FIGS. 19–27. Nuclei showing number and arrangement of chromatic bodies. FIG. 28. Nucleus in position to divide between daughter pyrenoids. FIG. 29. Cell stained with Heidenhain's iron-alum haematoxylin. Stage in revolution of protoplast, pyrenoid constricting.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regions are seen in surface view, the pyrenoid occasionally appears segmented or compound (fig. 3); however, the lighter blue central region is still visible. When a living cell is treated with iodine the outermost portion of the pyrenoid gives a starch-like reaction. Timberlake (1901) found that in *Hydrodictyon* tangential segments are cut off successively from the pyrenoid and that these segments are transformed into starch. G. M. Smith (1914) observed that one or more portions of the pyrenoid of *Scenedesmus* show a transitional staining reaction, which indicates that the pyrenoid may form several segments simultaneously and more than one starch grain at a time. It is possible that the dark-staining regions observed in the pyrenoids of *Carteria* stained with crystal violet–iodine or (in living cells) with iodine represent stages in the transformation of the pyrenoid substance into starch as reported by Timberlake for *Hydrodictyon*, Smith for *Scenedesmus*, and Bold (1931) for *Chlorococcum*.

Living cells which have undergone a period of growth since the last preceding division contain numerous shining oval bodies (fig. 1). Although these bodies resemble the starch grains about the pyrenoid in their refractive behavior, they do not give a starch reaction when living cells are treated with iodine. In preparations stained with crystal violet–iodine the cytoplasm contains many small, somewhat angular purple starch grains (fig. 2). These grains, the so-called "stroma starch," resemble the starch grains immediately about the pyrenoid in their reaction to crystal violet–iodine and in their angular shape. There seems to be little doubt that the oval bodies seen in the living cell, which, except for their failure to stain with iodine, resemble starch, are the bodies that are stained with crystal violet–iodine. It is possible that the "stroma starch" grains have changed chemically after their formation and hence do not give the typical starch reaction when living cells are treated with iodine. Frequently two layers of starch grains, instead of one, surround the pyrenoid (fig. 2), and the "stroma starch" tends to be concentrically arranged throughout the cell with respect to the pyrenoid. These facts may indicate that in *Carteria*, as in *Hydrodictyon*, all the starch grains within the cell have been formed from the pyrenoid, each layer in turn being forced away from the pyrenoid by the formation and separation of successive new layers.

Small rounded mitochondria appear in sections stained with Heidenhain's haematoxylin. They are imbedded in the cytoplasm, and often each appears to be surrounded by a very narrow clear region (figs. 4, 5, 6). Numerous cytoplasmic inclusions are visible in preparations of whole cells, but because of the thickness of the cell and the variety of intracellular bodies present identification of them has proved impossible.

The pigment spot is invisible in fixed material stained with either haematoxylin. When living cells are treated with iodine the pigment spot gives a

starch-like reaction. It is possible that the pigment spot is stained with crystal violet–iodine, but if so it cannot be distinguished from the stroma starch grains. Franzé (1893) concluded that the pigment spot in the Chlorophyceae is composed of a number of highly refractive bodies of the nature of starch. Mast (1927) found that in unicellular members of the Volvocales the pigment spot consists of a spoon-shaped structure and a hyaline mass, the latter containing the photosensitive substance. Until the chemical nature of the substances composing the pigment spot is better known, no explanation can be offered for the starch-like reaction observed.

In fixed material stained with Heidenhain's or Ehrlich's haematoxylin, two blepharoplasts are visible, located just within the wall beneath the anterior papilla (figs. 8, 10). Occasionally a third one can be observed (fig. 9). Rarely, what appears to be a fourth blepharoplast is visible, but because of their arrangement in a single transverse plane, all four cannot often be seen at once. No cross sections were obtained which were certainly at the blepharoplast level.

When living cells are treated with iodine the contractile vacuoles increase in size, and the narrow layer of cytoplasm between them appears as a strand somewhat similar to the rhizoplast which has been figured for such forms as *Polytoma uvella* (Dangeard 1900, 1901; Entz 1913, 1918), *Chlamydomonas nasuta* (Kater 1929), *Haematococcus pluvialis* (Elliott 1934), and others. A similar layer of cytoplasm appears in cells fixed *in toto* and in longitudinal sections stained with either Heidenhain's or Ehrlich's haematoxylin (figs. 7, 8, 9, 10). In no case is it possible to trace this layer beyond the inner margins of the vacuoles. It seems clear that in *Carteria crucifera* a rhizoplast does not connect the nucleus with the blepharoplasts, and that the apparent strand in question is the layer of cytoplasm between the contractile vacuoles.

The nucleus is usually flattened and broader on its anterior side, which lies in contact with the pyrenoid. Posteriorly it is rounded and occasionally slightly elongate (figs. 9, 19, 21). In preparations subjected to Feulgen's nucleal reaction neither the nuclear membrane nor the nucleolus stains. The position of the nucleolus is indicated in such preparations by a colorless spherical or slightly elongated region in the center of the nucleus (figs. 19–28). The chromatin granules, stained purple or dark blue, form a sparse reticulum; occasionally the granules are concentrated or massed together and have the appearance of a large node (fig. 19). They are more numerous just within the nuclear membrane and about the nucleolus. The most conspicuous feature of the nucleus is the presence of one or more bodies, staining like chromatin, which are much larger than the granules obviously of chromatin. When a single body of this type is present, it may lie just within the nuclear membrane (fig. 20), beside the nucleolus (fig. 19), or at any

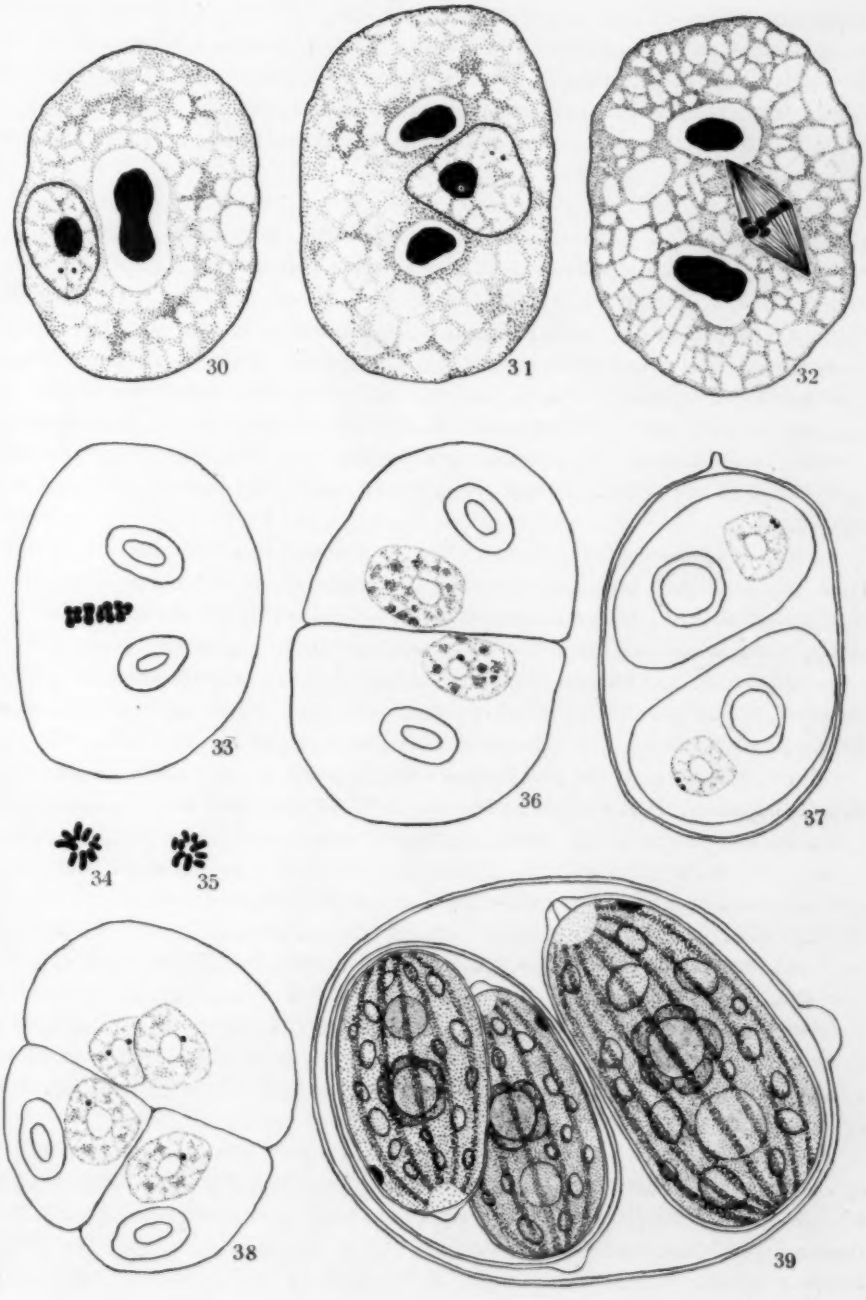
point between nuclear membrane and nucleolus (fig. 21). If two bodies are present they may be so close together as to suggest a recent division of a single body (fig. 22); one may be just within the nuclear membrane and the other near the nucleolus (fig. 23); or they may be variously located (fig. 24). Frequently from one to three additional smaller bodies appear, likewise variously located (figs. 25, 26, 27). These smaller bodies stain in the same way as the chromatin granules and the two larger bodies described above, but they can be observed most readily in lightly stained preparations and in a cell which is not the product of a recent division.

In preparations stained with Heidenhain's or Ehrlich's haematoxylin the nucleolus and nuclear membrane are visible. The chromatin granules form a sparse network between the nucleolus and the nuclear membrane and are more numerous just within the membrane and immediately about the nucleolus. Here, also, from one to five bodies variously located can be observed (figs. 8–18). There seems little doubt that the bodies which stain darkly with the Feulgen technique and with either haematoxylin stain are the same, since they are identical in number, size, and position. In haematoxylin preparations the nucleolus obscures from view anything above or below it, and the numerous cytoplasmic inclusions often make it difficult to determine whether a particular body is in the nucleus or in the cytoplasm. Since neither the nucleolus nor any cytoplasmic structure is stained by the Feulgen method, it is not surprising that four or five bodies should be observed more frequently with this method than with the haematoxylin stains.

The cell undergoes a period of growth before dividing, sometimes becoming so large and thick that it is difficult to determine the position of the nucleus and the pyrenoid. The cell either withdraws or loses its flagella. The protoplast revolves through an angle of 90° so that the anterior clear zone comes to lie in contact with the former equatorial region of the cell wall. In living cells the occurrence of this revolution can occasionally be recognized from the changed position of the clear region and the contractile vacuoles. In fixed preparations, stages in the revolution of the protoplast can sometimes be identified by the position of the blepharoplasts, by the enlarged contractile vacuoles, and by the layer of cytoplasm between the vacuoles (fig. 29). The pyrenoid divides by constriction either before the revolution

Explanation of figures 30–39

FIGS. 30–32. Cells stained with Heidenhain's iron-alum haematoxylin. FIG. 30. Nucleus in position to divide, pyrenoid constricting. FIG. 31. Nucleus in position to divide between daughter pyrenoids. FIG. 32. Lateral view of equatorial plate. FIGS. 33–38. Cells stained by Feulgen's nucleal reaction. FIG. 33. Lateral view of metaphase. FIGS. 34, 35. Polar views of equatorial plates. FIG. 36. Cell just after division, showing tightly appressed daughter protoplasts. FIG. 37. Two-celled colony. FIG. 38. Second division of colony just completed. FIG. 39. Palmella-like colony drawn from life.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of the protoplast or after the revolution is completed (figs. 29, 30). In consequence, a daughter pyrenoid is located toward each end of the cell and the nucleus lies between the daughter pyrenoids (figs. 28, 31). The increased size of the cell and the position of the nucleus between the daughter pyrenoids are the most satisfactory criteria for recognizing a cell which is about to divide.

A few cells were observed which contained two or even three pyrenoids (fig. 10) but which had not become enlarged and showed no indication of a revolution of the protoplast. One cell contained one pyrenoid and two nuclei, both located in the posterior part of the cell. Another cell possessed three pyrenoids in its central region and two nuclei in the posterior part. Neither cell had enlarged nor had its protoplast revolved. Although two pyrenoids are typically found only in cells which are about to divide, it appears that the pyrenoid can divide at any time to form two or three bodies. Also the nucleus may divide without a preceding revolution of the protoplast or division of the pyrenoid, and nuclear division is not always followed by cell division.

A nucleus which is preparing to divide is enlarged and usually elongate (figs. 28, 30). Such a nucleus resembles in structure a resting nucleus, and the darkly staining bodies previously described are still visible (figs. 28, 29). As the nucleus enters the prophases the chromatin granules appear more numerous and massed together, and it soon becomes impossible to distinguish the larger bodies observed in the resting nucleus. The nucleolus disappears and definitely organized chromosomes gradually appear.

Nine chromosomes are visible in a late prophase or in polar views of the equatorial plate. Three of these are about three times as long as wide and often curved, three about twice as long as wide, and three only slightly longer than wide (figs. 34, 35). In a lateral view of an equatorial plate the chromosomes appear short and somewhat bean-shaped (fig. 32).

The sharply pointed spindle lies either longitudinally or obliquely oriented between the daughter pyrenoids in the middle of the cell (fig. 32). The smallness of the division figures prevents the determination in lateral view of whether there are fibers extending from pole to pole in addition to those running from poles to chromosomes. There is no indication of a body of any nature at either pole of the spindle in Feulgen preparations (fig. 33) or with either of the haematoxylin combinations used (fig. 32).

The daughter chromosomes separate and move toward the respective spindle poles. After they reach the poles they become less distinct and appear massed or clumped. In connection with each daughter group the nuclear membrane and nucleolus appear. In Feulgen preparations the chromatin granules appear large and closely packed; there is little indication of a reticulum (fig. 36). This condition persists for some time.

The protoplast divides by constricting between the newly formed daughter nuclei. Although this appears to be a transverse division, it divides the protoplast longitudinally because of the previous revolution.

The cell may quickly divide a second time, or the two daughter protoplasts may develop walls while still within the mother-cell wall. The daughter cells shift positions within the mother-cell wall and usually come to lie with their anterior ends oppositely placed (fig. 37). They develop flagella which can be seen to be curled around each daughter cell and beating slightly. The daughter cells are freed by a local disintegration of the mother-cell wall.

If a second division follows, the daughter nuclei formed by the first division do not assume a typical resting condition, and the daughter cells do not form visible walls but remain tightly appressed. The second division is similar to the first, but it is more difficult to follow because of the crowding of the nuclear contents within a smaller space. It is uncertain whether a revolution of the daughter protoplasts occurs. The two nuclei do not always divide simultaneously; one may be at the equatorial-plate stage while the other is in an early prophase. The two daughter cells divide in planes at right angles to each other and to the plane of the first division.

A third division also may follow, or the four daughter protoplasts formed by the second division may develop walls. In the latter case the cells shift within the mother-cell wall and become variously arranged. The mother wall breaks down and the cells are liberated. If a third division follows the second, it is very similar to the first and second divisions, but the nuclei and pyrenoids are usually so close together that the story cannot be followed in detail. Eight small daughter protoplasts are finally formed, which develop walls and flagella. The mother wall breaks down and liberates the cells of the colony.

It was impossible to follow the blepharoplasts after the revolution of the protoplast was complete or to observe their formation and the growth of the flagella. Few colonies of sixteen cells were observed. Gametes were obtained only once during the course of this study. All later attempts by varying the nutrient concentration, illumination, and temperature failed to induce their formation. Either environmental conditions have not been suitable or else the strain is heterothallic. When gametes were formed the cultures were being watered with lake water, and it is possible that the second strain may have been introduced with the water.

Palmella-like colonies, consisting of several colonies within a mother wall, are occasionally formed in cultures growing on agar. These colonies are never very large, and the walls do not become noticeably gelatinous (fig. 39). When such colonies are placed on sand, the colonial walls break down and the enclosed daughter cells develop flagella and become free-swimming.

DISCUSSION

The neuromotor apparatus. Most investigators who have described blepharoplasts in the Volvocales report the presence of either one or two. Even quadriflagellate species seem generally to possess but two. In *Carteria crucifera*, however, certainly three and in all probability four blepharoplasts are present.

A cytoplasmic strand extending from the bases of the flagella toward the nucleus was described by Dangeard (1898) in *Phacotus angulosus*, *Chlorogonium euchlorum*, *Cercidium elongatum*, and *Chlamydomonas variabilis*. Later (1900, 1901) he referred to a similar structure in *Polytoma uvella* as a "rhizoplast." Rhizoplasts, connecting the blepharoplasts with the nucleus, have been described by Prowazek (1901) and Entz (1913, 1918) in *Polytoma uvella*, by Kater (1929) in *Chlamydomonas nasuta*, by Elliott (1934) in *Haematococcus pluvialis*, by Hovasse (1937) in *Eudorina* (*Pleodorina*) *illinoisensis*, and (somewhat doubtfully) by Hartmann (1919) in *Chlorogonium elongatum* and by Kater (1925) in *Polytomella citri*. Two rhizoplasts, one extending from each blepharoplast toward the nucleus, were reported by Mast (1916) for *Eudorina* sp. and by Hartmann (1921) for *E. elegans*.

In *Carteria crucifera* a strand which might be taken for a portion of a rhizoplast is visible between the contractile vacuoles. This strand, however, cannot be traced beyond the inner margins of the vacuoles. It seems clear that what is seen here is the layer of cytoplasm between the contractile vacuoles. This is the only species thus far investigated in which the nucleus is posterior to the pyrenoid. It is possible that in forms in which the nucleus is anterior, the narrow layer of cytoplasm between the vacuoles, erroneously interpreted as a rhizoplast, has been thought to extend to the nucleus.

An intranuclear centrosome whose division products appear at the poles of the spindle has been reported by Prowazek (1901) and Entz (1913, 1918) in *Polytoma uvella*, by Hartmann (1904) in *Volvox globator*, by Zimmermann (1921) in *V. aureus*, and by Kater (1929) in *Chlamydomonas nasuta*. Chatton (1910) described a centrosome within the nucleolus (karyosome) in *Pleodorina californica*. Elliott (1934) found an extranuclear centrosome which functions as a division center in *Haematococcus pluvialis*. Centrosomes were observed at the poles of the spindle by Aragão (1910) in *Polytomella agilis* and by Hartmann (1919) in *Chlorogonium elongatum*. Kater (1925) found no centrosomes in the resting nuclei of vegetative cells of *Polytomella citri*, although centrosomes appeared at the poles of the spindle. In encysted cells, however, extranuclear centrosomes were visible. In all these species the centrosomes and blepharoplasts, commonly connected by a rhizoplast, are reported to function in their distinctive capacities. In *Eudorina* (*Pleodorina*) *illinoisensis* according to Hovasse (1937), the two

blepharoplasts migrate from the bases of the flagella to the nucleus, move along the nuclear membrane to opposite sides of the nucleus and determine the respective poles of the spindle. After division each body moves to the anterior end of its daughter cell and divides to form two blepharoplasts. This is the only example among the Volvocales of a body reported to function as both centrosome and blepharoplast. Hovasse saw in the nucleus of this species from one to three bodies which are connected with the nucleolus by strands. He suggested that they be called "satellites of the nucleolus."

No bodies were observed at the poles of the spindle by Dangeard (1898, 1900, 1901) in several species of *Chlorogonium*, *Cercidium*, *Lobomonas*, *Phacotus*, *Chlamydomonas*, and *Carteria*, and in *Polytoma uvella*, by Merton (1908) in *Pleodorina illinoisensis*, by Reichenow (1910) in *Haematococcus pluvialis*, by Jameson (1914) in *Parapolytoma satura*, by Doflein (1916, 1918) in *Polytomella agilis*, or by Bělař (1921) in an unidentified species of *Chlamydomonas*.

In *Carteria crucifera* from one to five rather conspicuous bodies are variously located within the nucleus. There is no evidence that they are connected with the nucleolus. The bodies are most consistently and easily observed in Feulgen preparations. Although they are stained by haematoxylin, the darkly stained nucleolus and the cytoplasmic inclusions sometimes make their recognition difficult. There is no indication of a body at either pole of the sharply pointed spindle. It is possible that in previous studies one of these intranuclear bodies has been identified as a centrosome and that two of them have been interpreted as the product of a recent division of a centrosome. The Feulgen technique has not previously been employed extensively in investigating members of the Volvocales. In view of their reaction to this technique, it is suggested that the structures in question are chromocenters and that they are similar to the bodies observed by Hovasse in *Eudorina* (*Pleodorina*) *illinoisensis*. The term "chromocenter" seems suitable, for these bodies appear to be definite chromatic regions which remain condensed and highly chromatic during the resting stage.

The nucleolus. A chromatin-containing nucleolus which fragments to form the chromosomes was described by Jameson (1914) in *Parapolytoma satura* and by Kater (1925) in *Polytomella citri*. Kater (1929) held that the cortical portion of the nucleolus of *Chlamydomonas nasuta* contains chromatin. The nucleolus of *Carteria crucifera* contains no chromatin. Feulgen preparations show the nucleolus as a non-chromatic spherical body surrounded by an accumulation of chromatin granules. It is possible that it was the aggregation of granules about the nucleolus which led Kater to his conclusion.

Mitotic phenomena. Hartmann (1919) described in *Chlorogonium elon-*

gatum the formation of a half-spindle, which is followed by the division of both centrosome and half-spindle. One half-spindle and its centrosome migrate along the nuclear membrane to the opposite side of the nucleus from the position of the sister centrosome and half-spindle. The same author (1921) found that in the first division of *Eudorina elegans* the spindle is formed in the typical fashion, but that in the second division of some cells a half-spindle appears whose behavior is similar to that seen in *Chlorogonium*. In *Carteria crucifera* spindle-formation is of the typical sort.

In *Polytoma uvella*, according to Entz (1913, 1918) the flagella and blepharoplasts persist during the early stages of division but eventually disappear. After mitosis a body buds off from the nucleolus, divides, and one division product remains in the nucleus as the centrosome, while the other migrates to the anterior end of the cell, the rhizoplast appearing behind it. At the anterior end it divides to form two blepharoplasts. Kater (1929) and Elliott (1934) found that in *Chlamydomonas nasuta* and *Haematococcus pluvialis* the flagella and blepharoplasts disappear during division. After the division is completed a body buds off from the centrosome and migrates to the anterior end of the cell, remaining connected with the centrosome by a rhizoplast. This body divides to form the two blepharoplasts. Aragão (1910) and Doflein (1916, 1918) report that in *Polytomella agilis* one blepharoplast and two flagella pass to each daughter cell during division. Each blepharoplast then divides and two additional flagella are formed in each daughter cell. According to Jameson (1914), in *Parapolytoma satura* the blepharoplasts and flagella pass during cell division to a single daughter cell. In the daughter cell which does not receive these structures, a body buds off from the nucleolus, divides, and the daughter halves migrate to the anterior end where they become blepharoplasts. Kater (1925) reported that in *Polytomella citri* the blepharoplasts persist and divide during the division of a vegetative cell. In an encysted cell a new centrosome and blepharoplasts are formed by a bud from the nucleolus which divides. One division product remains outside the nucleus, supposedly as the centrosome, while the other migrates to the anterior end of the cell and divides to form the blepharoplasts. Hovasse (1937) found that in *Eudorina* (*Pleodorina*) *illinoisensis* the two blepharoplasts, after cell division, migrate from the poles of the spindle to the anterior ends of the respective daughter cells and divide.

In *Carteria crucifera* it was not possible to follow the blepharoplasts after the revolution of the protoplast was completed. No stages were observed which indicated the budding off of a body from the nucleolus. The blepharoplasts do not stain with Feulgen's nucleal reaction; they seem to have no relation to the bodies present in the nucleus.

Kater (1929) observed a revolution of the protoplast before mitosis through an angle of 90° in *Chlamydomonas nasuta*. A similar revolution occurs in *Carteria crucifera*.

Dangeard (1898) reported the following chromosome numbers: *Chlamydomonas Dilli* 10, *C. variabilis* 10, *C. monadina* 30, *Carteria cordiformis* 12. Pascher (1916) found 10 chromosomes in an unidentified species of *Chlamydomonas*. Eight chromosomes are figured by Bělař (1926) in an unnamed *Chlamydomonas*. Moewus (Hartmann 1934) observed 10 in *Chlamydomonas eugametos*, in *C. paupera*, and in a hybrid between *C. eugametos* and *C. paupera*. *Carteria crucifera* has nine. There is apparently no relation between the number of chromosomes and the number of flagella in *Chlamydomonas* and *Carteria*. Species of the biflagellate *Chlamydomonas* thus far investigated have chromosome counts ranging from eight to thirty. The two species of the quadriflagellate *Carteria* which have been studied have counts of nine and twelve chromosomes.

SUMMARY

The chloroplast of *Carteria crucifera* is H-shaped in longitudinal section; strands extend from the periphery to the central region where the pyrenoid is located.

The outermost portion of the pyrenoid at times stains differently from the remainder; this differentiated portion may represent a transition stage in the transformation of the pyrenoid substance into starch. The occurrence of two layers of starch grains close about the pyrenoid and the variation in staining reaction of the pyrenoid indicate that from it are formed all the starch grains within the cell.

Three, occasionally four, blepharoplasts are visible at the bases of the flagella. In fixed material the contractile vacuoles are enlarged and the layer of cytoplasm between them resembles a rhizoplast. In no case is it possible to trace this layer or strand beyond the inner margins of the contractile vacuoles.

The nucleus has a distinct membrane, a large nucleolus, and chromatic material in the form of strands and knots or granules. The nucleolus contains no chromatin. Within the nucleus are from one to five variously located bodies which are larger than the granules obviously of chromatin, yet which stain like chromatin with Feulgen's nucleal reaction and with haematoxylin. It is suggested that these bodies are chromocenters.

Nuclear division is preceded by a revolution of the protoplast through an angle of 90° and by the division of the pyrenoid by constriction. There is no indication of a centrosome at either pole of the spindle, which lies longitudinally or obliquely between the daughter pyrenoids. The protoplasm divides by constriction.

There is apparently no relation between the number of chromosomes and the number of flagella. *Carteria crucifera* has nine chromosomes.

WARREN, ILLINOIS

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BIOTIN AND THE GROWTH OF FUSARIUM AVENACEUM[1]

WILLIAM J. ROBBINS AND ROBERTA MA

(WITH SIX FIGURES)

Vitoria (21) reported in 1939 that *Fusarium avenaceum* did not grow in a liquid medium containing per liter 7.2 g. cane sugar, 3.6 g. dextrose, 1.23 g. $MgSO_4 \cdot 7\,H_2O$, 2.72 g. KH_2PO_4, and 2.02 g. KNO_3, but grew on the same medium solidified with agar. Other species of *Fusarium* grew well in the liquid medium. Vitoria found further that the addition to his medium of the filtrate from a six-day-old culture of *Penicillum sp.* permitted the development of *F. avenaceum*. As little as 0.25 ml. of the *Penicillum* filtrate was effective though the benefit increased with additions up to 50 ml. of filtrate.

The nitrogen in the basic medium used by Vitoria was in the form of nitrate, which is a poor source for some fungi (14). It might be suggested that the benefit observed from the filtrate of the *Penicillum* culture was due to organic nitrogen derived from that fungus. However, 0.25 ml. of the filtrate must have contained very little organic nitrogen. This consideration suggested that the failure of *F. avenaceum* to grow in the liquid medium might have been the result of growth substance deficiencies which were supplied by the agar or by the *Penicillum* filtrate. One of the authors has been interested in the growth substances in agar (13, 16) and the effect of agar on *F. avenaceum* seemed worthy of further investigation.

MATERIALS AND METHODS

Three strains of *F. avenaceum* were obtained. One was kindly supplied by Dr. E. C. Stakman; it will be referred to as strain (M). The second was secured through the courtesy of Dr. R. W. Goss and will be called strain (N). The third was obtained through the kindness of Dr. Vitoria and will be called strain (V). The three strains differed in appearance of colony, pigment production, and size and shape of conidia. The (M) and (N) strains were much alike and both produced conidia which were from 1 to 3 celled and inclined to be straight with blunt ends. The (V) strain formed reddish pigment freely and the conidia were from 5 to 7 celled, curved, and with pointed ends.

Three basic media were used as follows:

Solution 1 contained per liter of distilled water 50 g. dextrose, 1.5 g. KH_2PO_4, 0.5 g. $MgSO_4 \cdot 7\,H_2O$, and 2.0 g. asparagine; solution 2 was Vi-

[1] Supported in part by a grant from the Josiah Macy Jr. Foundation.

toria's liquid medium; solution 3 was solution 1 with 1.76 g. of $(NH_4)_2SO_4$ instead of asparagine. This quantity of $(NH_4)_2SO_4$ contained the same amount of nitrogen as the 2 g. of asparagine.

The dextrose was Corn Products C.P.; the asparagine was purified by treatment with Norit A and recrystallization from alcohol; the other chemicals were of C.P. grade unless specifically noted. All glassware was pyrex, cleaned with chromic acid cleaning mixture and thoroughly rinsed with tap

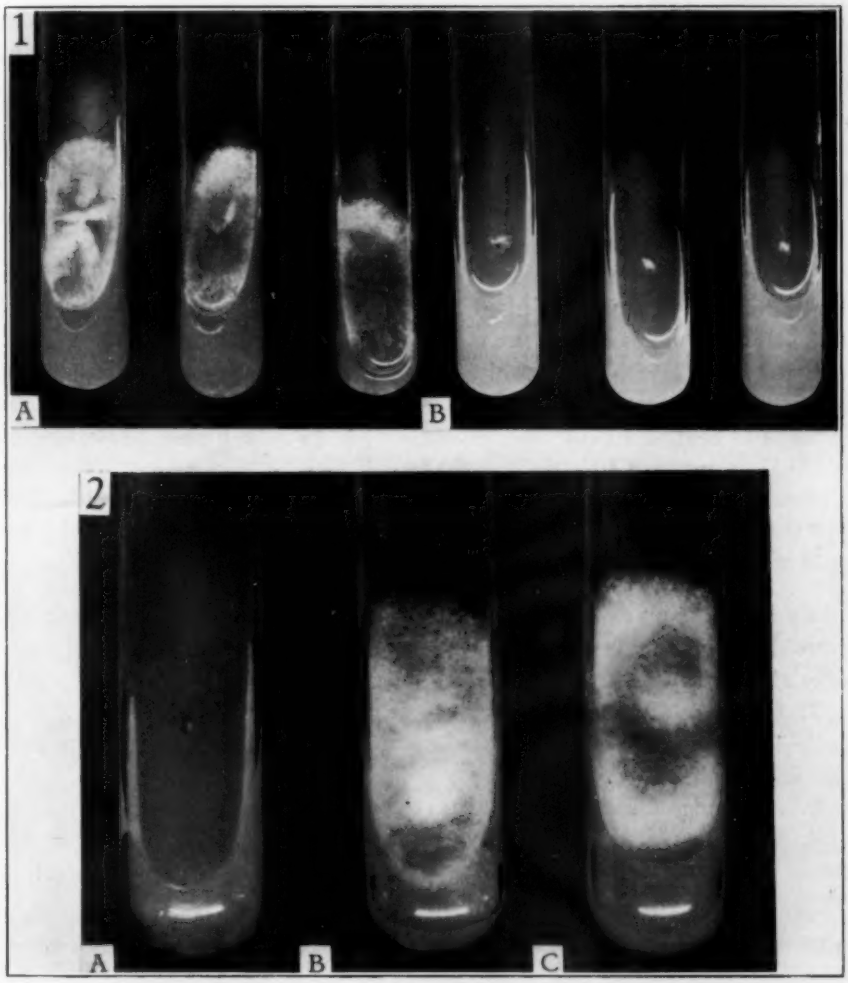

FIG. 1. *F. avenaceum*, strain (V), on solution 2 plus (A) 1.5% Difco agar (B) 1.5% purified Difco agar. Age of cultures 3 days. FIG. 2. Three strains of *F. avenaceum* on solution 2 plus 1.5% purified Difco agar: A, strain (V); B, strain (N); C, strain (M). Age of cultures 4 days.

and distilled water. Media were sterilized at 12 pounds pressure for 20 minutes. For inoculation a bit of mycelium approximately a square mm. in area was removed from a culture on agar; care was taken to transfer none of the agar. In some instances conidia instead of mycelium were used for inoculation. Cultures were incubated at 25° C. in the dark.

EXPERIMENTS

Growth in liquid cultures. Vitoria's report that his strain of *F. avenaceum* does not grow in his liquid medium was confirmed. Triplicate cultures containing 25 ml. of solution 2 in 125 ml. Erlenmeyer flasks were inoculated with bits of mycelium from a culture of strain (V) on a thiamin-peptone agar (18). Growth in a period of 7 days was slight and subcultures into solution 2 failed completely. The same results were secured with solution 1 in which the nitrogen was supplied as asparagine.

Strain (M) grew well in solution 1, in which it produced a greenish yellow pigment. It was carried for four successive passages in this solution and showed no evidence of reduced growth in the fourth passage. Similar results were secured with solution 2, though the growth was not so vigorous as in solution 1 and no pigment was produced.

Strain (N) grew well in solution 1 through four successive passages but produced less pigment in the solution than strain (M). Its growth was as vigorous as that of strain (M). Strain (N) grew in solution 2 also but less well than in solution 1.

From this experiment it appeared that the three strains differed in their growth responses and that a solution in which strain (M) and (N) grew quite well was unsatisfactory for strain (V).

Effect of agar. Solution 2 was solidified with 1.5 per cent agar and approximately 8 ml. placed in test tubes, sterilized, and sloped. Triplicate tubes of four types of agar were used as follows:

1. A sample of Difco standardized granulated agar.
2. The same agar purified by extraction with 5 per cent aqueous pyridine and alcohol.[2]
3. A sample of Eimer and Amend flake agar.
4. The same agar purified by extraction with pyridine and alcohol.

Strain (V) grew well on solution 2 plus 1.5 per cent Difco agar; the myce-

[2] The agar was purified as follows: 1 pound of agar was treated in a percolator with 12 liters of 5 per cent aqueous distilled pyridine; the final percolate was colorless. The agar was then washed with 2 liters of ethyl alcohol and boiled with 12 liters of ethyl alcohol. It was filtered through a Buchner funnel and dried at 50–60° C. The percolate and washings were combined, evaporated to remove the pyridine and alcohol, and made up to volume with distilled water so that 1 ml. of the extract was equivalent to 1 gram of the extracted agar.

lium nearly covered the surface of the slope in three days. It grew little or not at all when the purified agar was substituted for the unpurified agar (fig. 1). Similar results were obtained with the purified and unpurified Eimer and Amend flake agar, though the organism grew less rapidly on the medium solidified with the unpurified flake agar than on that in which unpurified Difco agar was used.

Strains (M) and (N) grew on the media solidified with all four types of agar (fig. 2), but the growth on media made with the purified agars was less rapid than on that made with either of the unpurified agars.

Subcultures of each strain on each type of agar were carried for three successive passages[3] with no significant differences between the growth on a particular medium in successive passages. The development of strain (M) was much like that of strain (N); white cottony aerial mycelium formed on the agar slopes and little pigmentation was observed. The aerial mycelium of strain (V) grown on the unpurified agars was less luxuriant than that of strain (M) and (N) and considerable pink color was formed.

Effect of agar extract on growth of strain (V). The failure of strain (V) to grow satisfactorily on media made with the purified agars was because of the removal of some beneficial principle from the agar in the purification. This was demonstrated by observing the effect on its growth of additions of the agar extract to the purified agar. Solution 2 with 1.5 per cent purified Difco agar was prepared. To some tubes extract[4] was added equivalent to 1 per cent, 5 per cent, or 15 per cent agar. The organism made little or no

TABLE 1

Growth of strain (V) of F. avenaceum on agar slopes of solution 2 plus 1.5% purified Difco agar to which various supplements were added. For details see text.

Additions to solution 2 plus 1.5% purified agar	Diameter of colonies in cm.	
	2 days	3 days
None	No growth	No growth
5 supplements	1.3	2.5
7 supplements	No growth	No growth
12 supplements	1.0	2.0
Extract equivalent to 1% agar	1.95	3.8
Extract equivalent to 5% agar	2.4	3.8
Extract equivalent to 15% agar	2.2	3.5
Biotin	1.3	2.5
Ca fraction	1.7	3.0
Dr fraction	No growth	No growth
Ca & Dr fraction	2.4	4.0

[3] The growth of strain (V) on the media made with purified agars was so poor in the first passage that no attempt was made to carry it beyond the second passage on the purified agar.

[4] The extract contained 10 per cent dry matter of which about 15 per cent was ash.

growth on the purified agar but grew well on the purified agar to which the agar extract was added (table 1). Rapid growth occurred in all tubes containing agar extract and in less than a week the slope was covered with mycelium and an orange-colored mass of spores occupied an area 1 cm. or more in diameter on those cultures with extract equivalent to 15 per cent agar (fig. 3). A similar but somewhat smaller area of spores was formed on the cultures with extract equivalent to 5 per cent agar. None was observed in the tubes with 1.5 per cent unpurified agar or on those containing purified agar and extract equivalent to 1 per cent agar.

Effect of various growth substances on strain (*V*). There appeared to be something extracted from the agar which induced growth of strain (V). This material or materials was not effective through modifying the pH since the hydrion concentration of media made with the purified and unpurified agar was found to be the same. It was thought not to be a mineral nutrient or to serve as a source of nitrogen because mineral salts and a nitrogen source were added to both the purified and unpurified agars and, further, the nitrogen compounds in agar are relatively unavailable. The effect of various growth substances was, therefore, determined.

The following media were prepared in tubes; six replications of each medium were used. The source of each growth substance and the quantity used per tube are given below:

1. Solution 2 with 1.5 per cent purified Difco agar.

2. Solution 2 with 1.5 per cent purified Difco agar plus thiamin, Merck's synthetic, 10 millimicromoles; pyridoxine, Merck's synthetic, 20 millimicromoles; calcium pantothenate, Merck's synthetic, 5 micrograms; biotin,[5] du Vigneaud, 0.004 microgram; nicotinamide, S.M.A., 10 micrograms.

3. Solution 2 with 1.5 per cent purified Difco agar plus para-aminobenzoic acid, Eastman, 5 micrograms; pimelic acid, Eastman, 5 micrograms; i-inositol, Pfanstiehl, 10 mgm.; riboflavine, Labco natural, 10 millimicromoles; glutamine, György, 10 micrograms; 2-methyl-l, 4-naphthoquinone (S.M.A.) 5 micrograms; ascorbic acid, Merck's natural, 5 micrograms.

4. Solution 2 with 1.5 per cent purified Difco agar plus the 12 growth substances.

Two days after inoculation there was no growth on medium 1, the colonies on medium 2 averaged 1.3 cm. in diameter, no growth appeared on medium 3 and on medium 4 the colonies averaged 1.0 cm. in diameter (table 1). At the end of a week very little growth had occurred on media 1 and 3; the surface of the agar slopes of media 2 and 4 was covered with mycelium though the growth was somewhat less vigorous on medium 4 than on medium 2.

[5] The biotin was a pure preparation of the methyl-ester supplied through the courtesy of Dr. Vincent du Vigneaud and described in a recent publication (5).

These results indicated that one or more of the five growth substances in the mixture used in medium 2 induced growth of strain (V) on solution 2 prepared with 1.5 per cent purified agar. Biotin methyl-ester was found to be the effective substance.

Effect of biotin on growth of strain (V). The growth of strain (V) on solution 2 solidified with 1.5 per cent purified agar plus 0.004 microgram of biotin per tube containing 8 ml. of medium was found to be about equal to that on the same medium plus pyridoxine, thiamin, calcium pantothenate, and nicotinamide in addition to the biotin. Growth in the tubes containing biotin was, however, less rapid than on media containing agar extract equivalent to 1 per cent agar (table 1).

These observations demonstrated that the growth of strain (V) on media prepared with the purified agar was poor because the organism suffered from a biotin deficiency. It appeared that sufficient biotin was present in the unpurified agar to satisfy the deficiency and that the biotin was removed from the agar by the method of purification used.

However, the addition of agar extract to the purified agar seemed to be more effective than the addition of the 0.004 microgram of pure biotin. Why should this be? Several possibilities must be considered. Perhaps the amount of biotin added in the extract equivalent to 1 per cent agar was greater than the 0.004 microgram of pure biotin used per tube in the experiment described above. *F. avenaceum* may exhibit partial deficiencies for growth substances other than biotin which are supplied by the agar extract. If so, the substances involved are not pyridoxine, pantothenic acid, thiamin, or nicotinamide, since growth with these four supplements in addition to biotin was about the same as with biotin alone. It is probable also that they are not included in the second group of seven since the growth when all twelve growth substances were used was not better than when the biotin alone was present (table 1). The minerals in the agar extract may have been important or some other factor may have played a role. Some of these possibilities were investigated.

Effect of quantity of biotin. Tubes of solution 2 with 1.5 per cent purified agar were prepared. To some tubes extract equivalent to 0.5 per cent, 1.0 per cent, 5 per cent, or 15 per cent agar was added; to others biotin was added in the following amounts per tube: 0.001, 0.005, 0.01, 0.05, or 0.1 microgram. Eight mgs. of asparagine were added to some tubes containing 0.05 microgram or 0.1 microgram of biotin. The experiment was performed in triplicate and carried for two successive passages. The second passage was started when the cultures in the first passage were three days old. Tubes of each kind of medium in the second passage were inoculated from the growth in tubes of the same kind of medium in the first passage.

TABLE 2

Growth of strain (V) of F. avenaceum on agar slopes of solution 2 plus 1.5% purified Difco agar to which various supplements were added.

Additions to 8 ml. of solution 2 plus 1.5% purified agar	Diameter of colonies cm.			
	1st passage		2nd passage	
	2 days	3 days	2 days	3 days
None	0.6	0.6	No growth	No growth
Extract equivalent to 0.5% agar	2.0	3.5	1.6	2.9
Extract equivalent to 1.0% agar	.....	.....	1.9	3.1
Extract equivalent to 5.0% agar	2.3	3.7	2.0	3.4
Extract equivalent to 15.0% agar	2.3	4.0	.....	.....
0.001 microgram biotin	0.6	1.1	No growth	0.4
0.005 microgram biotin	2.0	3.3	1.5	2.7
0.01 microgram biotin	2.0	3.1	1.4	2.5
0.05 microgram biotin	1.9	3.5	1.4	2.3
0.1 microgram biotin	1.9	3.3	1.5	2.5
0.05 microgram biotin and 8 mgm. asparagine	1.6	2.9	1.2	2.1
0.1 microgram biotin and 8 mgm. asparagine	1.5	2.7	1.3	2.3

In the first passage there was some growth at the expense of the original inoculum on the purified agar, but little or no growth occurred in the second passage (table 2). The effect of 0.001 microgram of biotin per tube was slight; 0.005 microgram produced considerable growth and as far as colony diameter was concerned there was little difference between the effects of 0.005 microgram and 0.1 microgram of biotin. The heaviness of growth, however, increased as the amount of biotin increased (fig. 4). The addition of asparagine reduced the rapidity of growth. Even with 0.1 microgram of biotin per tube the diameter of the colonies was less than with extract equivalent to 1 per cent agar. This suggested that although biotin is the material primarily responsible for the effectiveness of agar in causing growth of strain (V) there are other soluble substances in the agar which favorably affect growth of the organism in the presence of biotin. This assumption is supported by comparing the growth *F. avenaceum* in liquid cultures containing pure biotin with that in cultures containing agar extract with known biotin content.

Quantity of biotin in Difco agar. The quantity of biotin in the extract of Difco agar was estimated by the growth of *Ashbya gossypii* as described by Robbins and Schmidt (15). Various quantities of the agar extract or of biotin (table 3) were added to 25 ml. quantities of the solution used by Robbins and Schmidt and the hydrion concentration was adjusted with

Na_2HPO_4 so that it was pH 5.6 after sterilization. The cultures were inoculated with a suspension of *Ashbya* and incubated at 25° C. for 15 days. Dry weights were determined by filtering into Gooch crucibles and drying at 100° C. The biotin was estimated by the curve given by Robbins and Schmidt. The experiment was performed in triplicate.

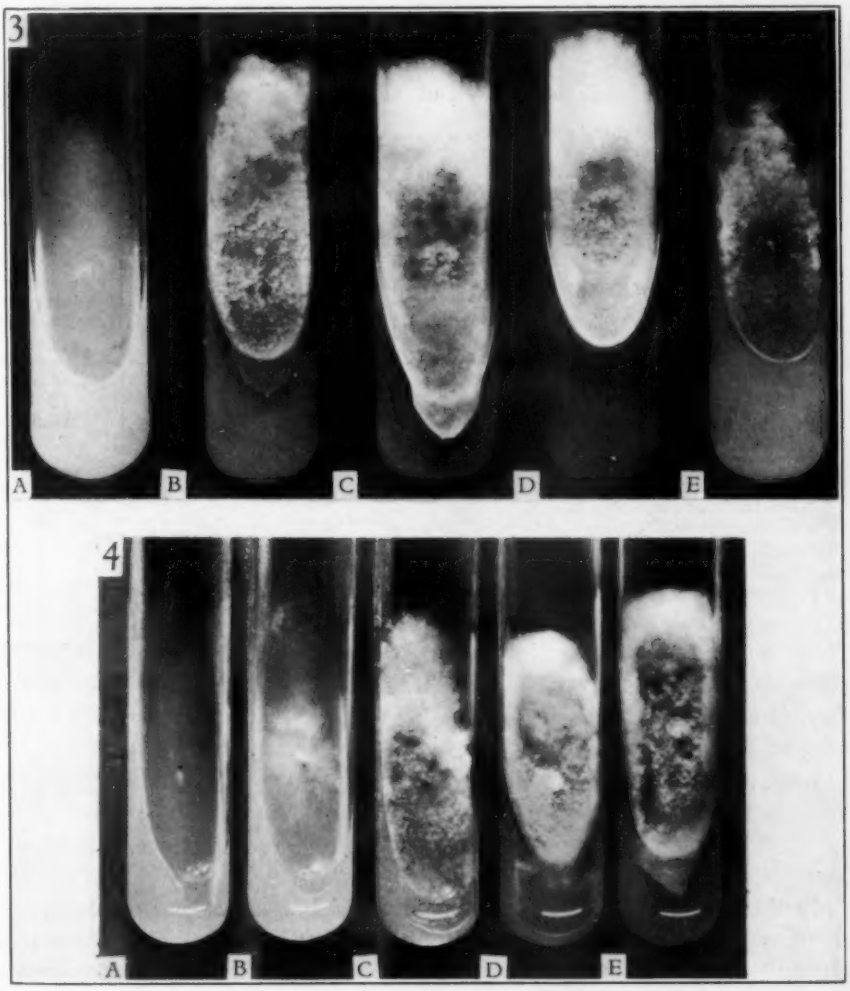

FIG. 3. *F. avenaceum*, strain (V), on solution 2 with 1.5% purified Difco agar plus (A) nothing, (B) extract equivalent to 1% agar, (C) extract equivalent to 5% agar, (D) extract equivalent to 15% agar, (E) 0.004 microgram biotin per tube. Age of cultures 6 days. FIG. 4. *F. avenaceum*, strain (V), on solution 2 with 1.5% purified Difco agar plus (A) nothing, (B) 0.001 microgram biotin, (C) 0.005 microgram, (D) 0.01 microgram, (E) 0.1 microgram per tube. Age of cultures 6 days.

Some growth occurred in the check solution probably at the expense of biotin carried over with the inoculum which came from an agar containing thiamin and peptone. However, 0.005 microgram of biotin increased growth and 0.01 microgram gave a further increase. A favorable effect of 0.025 ml. of agar extract containing 2.5 mg. dry matter was observed and further benefits were obtained up to 0.625 ml. of agar extract per flask. Growth was less with 1.25 ml. of agar extract per flask than with 0.625 ml., evidently because of some inhibitory action of this quantity of material.

In the fourth and fifth columns of table 3 the quantity of biotin in the

TABLE 3

Growth of Ashbya gossypii in solutions supplemented with agar extract or pure biotin and the quantity of biotin in the agar extract as estimated from the growth of Ashbya.

Additions to 25 ml. of Robbins and Schmidt solution	Av. dry wt. Ashbya per culture mg.	Net dry wt. Ashbya per culture mg.	Biotin per culture milli-micrograms	Biotin per ml. agar extract milli-micrograms
1.25 ml. agar extract	20.3	15.5	.....	.....
0.625 ml. agar extract	41.0	36.2	58	92.8
0.25 ml. agar extract	24.5	19.7	24	96.0
0.125 ml. agar extract	16.9	12.1	9.9	79.9
0.025 ml. agar extract	10.3	5.5	2.8	112.0
None	4.8	.....	.....	.....
0.005 microgram biotin	12.8	8.0	.....	.....
0.01 microgram biotin	15.2	10.4	.....	.....

agar extract added per culture and the quantity of biotin per ml. of agar extract as calculated from the growth of *A. gossypii* are given.

The average amount of biotin per ml. of agar extract as estimated by this means was 0.095 microgram. Since 1 ml. of the extract was equivalent to 1 g. of original agar the biotin content of the original agar also was 0.095 microgram or 1 g. of biotin in about 10,526 kg. of agar.[6]

Kögl and Tonnis (8a) estimated 1 g. of biotin in 3,125 kg. of dried egg yolk. On the basis of these figures the Difco agar we used contained about one-third as much biotin as dried egg yolk, the source from which Kögl and Tonnis originally isolated it.

In the experiments described above in which agar extract or biotin was added to pure agar it was observed that the growth in 8 ml. of medium containing 1 per cent agar extract was somewhat better than in the same quantity of medium plus 0.004 microgram of biotin. Eight ml. of 1 per cent agar extract contained 0.08 ml. of agar extract, or 0.0076 microgram of biotin, a quantity sufficient to give the results observed.

[6] Du Vingneaud, using growth of yeast for bio-assay, found the biotin content of this agar extract to be 0.041 microgram per ml., about one-half the value we obtained from the growth of *Ashbya*. A sample of Eimer and Amend flake agar contained about one-twentieth as much.

Effect of biotin or agar extract in liquid cultures. That a biotin deficiency is responsible for the failure of the (V) strain of *F. avenaceum* to grow in liquid culture lacking organic substances other than sugar and that its biotin content is primarily but not entirely responsible for the beneficial effect of agar was further demonstrated by the following:

The (V) strain was grown in 25 ml. quantities of solution 2, solution 2 plus 0.01 microgram of biotin per flask, solution 3, and solution 3 plus 0.01 microgram of biotin per flask. A suspension of spores from a culture on solution 2 solidified with 1.5 per cent purified Difco agar plus extract equivalent to 15 per cent agar was used as inoculum. The experiment was performed in triplicate. The spores germinated but very little mycelium developed in the solutions without biotin. Considerable growth occurred in the solutions with biotin; the growth in solution 3 was somewhat better than in solution 2.

TABLE 4

Growth of F. avenaceum in solutions supplemented with pure biotin, with agar extract, or with pure biotin and mineral supplements. Age 7 days.

Additions to 25 ml. of solution 3	Amt. biotin in agar extract det'd by growth of *Ashbya* microgram	Amt. biotin in agar extract det'd by du Vigneaud microgram	Av. dry wt. mycelium per culture mg.
None	.....	.....	No growth
0.001 microgram biotin	.....	.....	1.4
0.005 microgram biotin	.....	.....	3.6
0.01 microgram biotin	.....	.....	6.7
0.05 microgram biotin	.....	.....	16.7
0.1 microgram biotin	.....	.....	21.7
0.0125 ml. agar extract	0.00119	0.0005	10.6
0.0250 ml. agar extract	0.00238	0.0010	11.8
0.0625 ml. agar extract	0.00595	0.0025	17.9
0.125 ml. agar extract	0.0119	0.0051	24.9
0.25 ml. agar extract	0.0238	0.0103	29.1
0.5 ml. agar extract	0.0476	0.0205	35.4
1.25 ml. agar extract	0.1190	0.0513	53.1
0.1 microgram biotin and mineral supplements	.....	.....	22.6

In a second experiment various amounts of biotin or agar extract (table 4) were added to 25 ml. quantities of solution 3 and the cultures inoculated with a suspension of spores as before. To one set of cultures containing 0.1 microgram of biotin per flask the following mineral supplements were added per liter: 0.027 p.p.m. B, 0.106 p.p.m. Cu, 0.800 p.p.m. Fe, 0.053 p.p.m. Ga, 0.053 p.p.m. Mn, 0.053 p.p.m. Mo, 0.479 p.p.m. Zn. The experiment was performed in quadruplicate. Dry weights were determined after 7 days incubation at 25° C.

The spores germinated but no weighable mycelium was obtained in the

cultures with no organic material except dextrose. In the flasks containing 0.001 microgram of biotin 1.4 mg. of mycelium was secured, and the amount of growth increased with the quantity of biotin supplied up to 21.7 mg. with 0.1 microgram of biotin. The addition of the mineral supplements to cultures containing 0.1 microgram of biotin made no significant improvement. This suggests that mineral supplements were not responsible for the superiority of agar extract as compared to pure biotin. The addition of 0.0125 ml. of agar extract (1.25 mg. dry matter) per flask permitted 10.6 mg. of mycelium to develop and the amount of growth increased with the amount of agar extract up to 53.1 mg. of mycelium with 1.25 ml. of agar extract per flask (table 4).

In the second column of table 4 the amount of biotin supplied by the agar extract per culture is given as calculated from the determinations in table 3, and in the fourth column as determined by du Vigneaud. It is evident that growth with the agar extract was greater than would be expected from its biotin content. For example, a yield of 17.9 mg. mycelium was obtained with 0.00595 microgram of biotin in the agar extract, while the yield with 0.005 microgram of pure biotin was but 3.6 mg.; 53.1 mg. of mycelium were obtained with 0.119 microgram of biotin in the agar extract while 0.1 microgram of pure biotin gave 21.7 mg.

Effect of Ca and Dr fractions. That other growth substances than biotin affect the growth of *F. avenaceum* is further supported by the action of two fractions obtained from potato extract as described earlier (17). The Ca fraction in the amount used was quite beneficial, the Dr fraction was ineffective and combined they gave greater growth than either alone and as great as or greater than the most favorable amount of agar extract (table 1). The Ca fraction probably contained biotin as well as other growth substances, since it is a charcoal eluate from an extract of potato tubers. The Dr fraction contained little or no biotin, since it is the filtrate from the charcoal-treated potato extract. It is of some interest to contrast the effect of these fractions on *F. avenaceum* and on *Phycomyces Blakesleeanus.* The latter organism was affected by the Dr fraction to a considerably greater degree than by the Ca fraction (17).

Mutation of strain (V) and biotin deficiency. In the course of our experiments it was observed that an inoculation from a subculture of strain (V) grew satisfactorily in solution 2 although inoculations from the parent culture made earlier into the same medium had not grown. This variant from strain (V) grew also on solution 2 solidified with purified Difco agar; on solution 2 solidified with 1.5 per cent Difco agar it grew more rapidly than strain (V) and had a less tufted and more uniform cottony white aerial

mycelium. Both produced considerable reddish pigment. A second variant appeared which grew on purified agar but was without pigment.

It was noted also that in one or two tubes of a half-dozen containing solution 2 and 1.5 per cent purified agar vigorous growth had occurred after a month, though for two weeks the inoculum had shown little growth and in the balance of the tubes the development was still slight after a month.

Furthermore, although on some media, for example those to which extract equivalent to 5 or 15 per cent agar had been added, the growth was uniform, on other media considerable local differences in color or character of mycelium appeared as the cultures aged.

The following explanations for these observations were considered.

The original culture of strain (V) might have been mixed, containing an organism with a biotin deficiency and others which were self-sufficient so far as that growth substance was concerned. This did not seem probable because of the difficulty of conceiving that the self-sufficient strains in such a mixture lay dormant on a biotin deficient medium for a week or more and then sprang into active growth, as occurred in some instances in old tubes containing solution 2 and purified agar. It was thought unreasonable also to expect several inoculations made from a culture of strain (V) to fail to grow in solution 2, which would mean that only the biotin-deficient element of the mixture had been transferred, while inoculations made from a subculture of the parent culture grew in the biotin-deficient medium.

It might be suggested that strain (V) became contaminated in the course of an experiment. This was considered unlikely because no other *Fusaria* than the three strains mentioned were in culture in the laboratory and most of the variants isolated from strain (V) were clearly different from strains (M) and (N) as well as (V).

It was thought also that strain (V) might possess a limited ability to synthesize biotin and that after two or three weeks on a biotin-deficient medium sufficient biotin might have been accumulated in the mycelium used as inoculum to permit growth to occur. However, the rapidity of growth once it started and the fact that subcultures from this growth were morphologically different from strain (V) made such an explanation untenable.

The fourth explanation considered was that strain (V) was unstable and produced saltations, dissociations, or mutations some of which were self-sufficient for biotin. Mutation is a well-known and common phenomenon in the *Fusaria*. Burkholder (3), Brown (2), Leonian (9), Sahai Vasudeva (19), Snyder (20), Orton (12), and others have contributed observations on its occurrence. The validity of this hypothesis was tested as follows:

Mono-conidial cultures. Two single-spore isolations from strain (V) were made. For this purpose a macro-conidium from a culture on solution 2

solidified with 1.5 per cent purified agar and containing extract equivalent to 15 per cent agar was used. Each mono-conidial isolation was grown on a thiamin-peptone agar. At intervals bits of mycelium were removed from each isolation and transferred to 5 slopes of solution 2 containing 1.5 per cent purified agar. The following results were obtained.

Five transfers from each monoconidial isolation were made to solution 2 containing 1.5 per cent purified agar when the cultures were two days old. These transfers grew slowly and by the end of 12 days the raised convoluted colonies were all of about the same size and averaged 1 cm. in diameter. By the end of 19 days three of the ten transfers showed evidence of a spreading surface growth and several were surmounted by white tufts but no colony had completely covered the agar slope. After a month eight of the ten had nearly covered the surface of the slope, the new growth consisting of reddish mycelium closely appressed to the agar. Transfers made from this later growth grew well on purified agar, indicating that a strain or strains self-sufficient for biotin had developed from the original biotin-deficient strain. Similar transfers were made from the monoconidial isolations when they were 4, 7, 9, 14, 21, and 28 days old, making a total of 70 transfers to purified agar from the monoconidial cultures on thiamin-peptone agar. One only of these 70 subcultures grew rapidly within the first week after transfer, which suggests that a mutation had occurred on the thiamin-peptone agar and had been transferred to the purified agar. Thirty-five of the remaining 69 cultures on the purified agar grew slowly for one, two, or three weeks and then grew rapidly, covering the surface of the agar slopes (fig. 5). Transfers to purified agar from the rapidly spreading mycelium of these cultures grew well, showing that mutation to a type capable of growing on a biotin-deficient medium had occurred (fig. 6). Transfers from the convoluted colonies which were growing slowly on the purified agar to the same medium either grew slowly or failed to grow. It is possible that all 69 of the transfers would have developed biotin-sufficient mutations if they had been observed for a longer period of time.

It is not possible to say that mutations occurred more frequently on the biotin-deficient medium, although the data cited above would seem to support that assumption. It should be remembered that on the biotin-deficient medium a mutation of strain (V) toward biotin sufficiency became evident because it spread over most of the agar surface. On a medium containing biotin a similar mutation would be overlooked and found by accident only. It is obvious that many questions on the type of mutation of *F. avenaceum* observed here remain to be studied.

The slow growth of strain (V) on the purified agar indicates either that biotin was not completely removed from the agar by the method of extraction employed or that the organism is able to synthesize a small and insuffi-

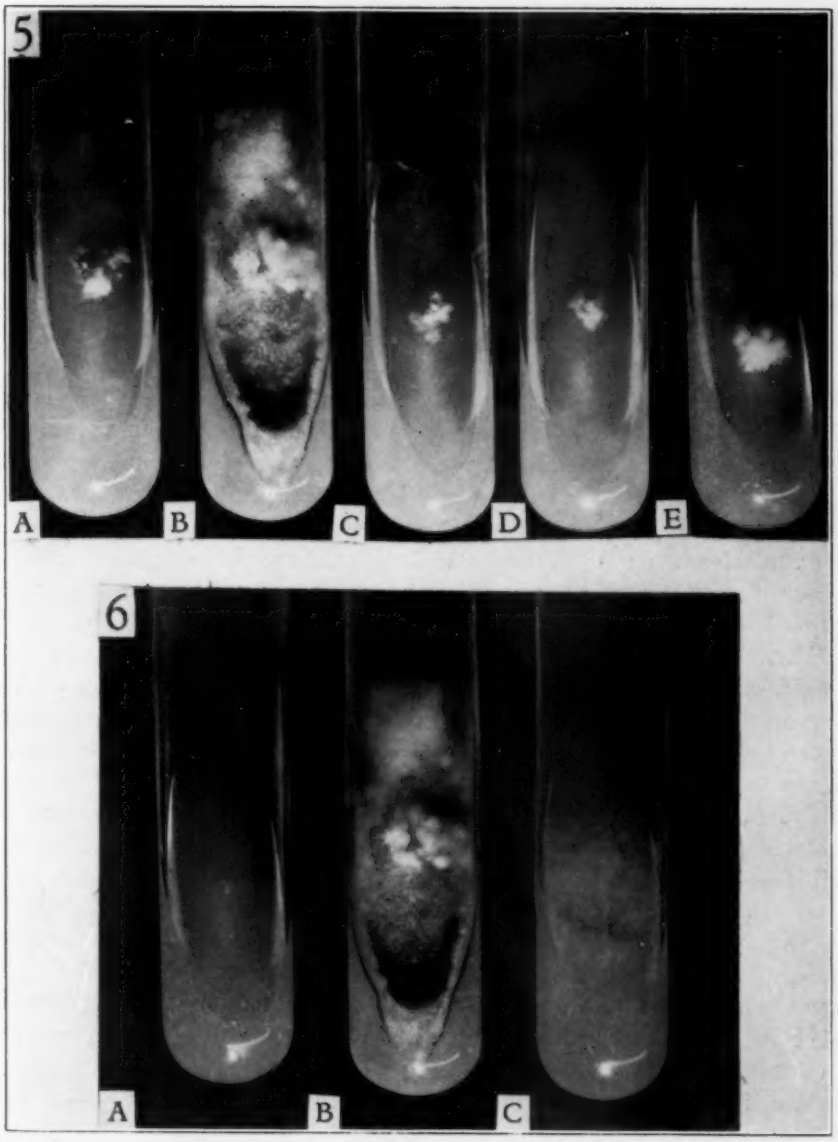

FIG. 5. Mutation of strain (V) to biotin-sufficient form. Five transfers from 9-day-old mono-conidial culture of strain (V) on thiamin-peptone agar to solution 2 plus purified agar. Age of transfers 12 days. Note that 4 of transfers have grown slowly and one has developed spreading growth over surface of agar. Transfer B resembled A, C, D, and E for first 7 days. FIG. 6. Cultures of strain (V) on solution 2 plus purified agar. B, mutating culture shown in figure 5. A, transfer from culture B when it was 2 days old before mutation developed; C, transfer from culture B when it was 9 days old after mutation had developed. Age of culture A was 7 days and of C was 3 days.

cient amount of the growth substance. The failure of the spores to do more than germinate in a liquid culture lacking biotin suggests that the former interpretation is perhaps correct.

DISCUSSION

Biotin deficiencies have been reported for a number of filamentous fungi including *Nematospora gossypii* (*Ashbya gossypii*) and *Lophodermium pinastri* (8), *Melanconium betulinum* and *Hypoxylon pruinatum* (4), *Marasmius androsaceus* and *Marasmius perforans* (11), *Melanospora destruens*, *Podospora curvula*, *Sordaria fimicola*, and *Sordaria sp.* (6). The work reported in this paper adds at least one strain of *Fusarium avenaceum* to this list.

Differences in growth substance deficiencies for strains of a given fungus also have been reported. Lindeberg (11) found one strain of *Marasmius perforans* to be biotin deficient while other strains of the same organism were autotrophic for that substance. Hawker (6) found some strains of *Melanospora destruens* to be biotin autotrophic while others were biotin deficient. Houston (7) reported one strain of *Corticium vagum* to require the addition of thiamin to the medium while others grew satisfactorily without its addition.

Mutations in the fungi resulting in a change in growth substance deficiencies are not unexpected but have not hitherto been reported, though Leonian and Lily (10) suspected the occurrence of such mutations in some of their experiments. It seems very probable that fungi differ considerably in their stability with regard to growth substance deficiencies. We have cultivated *Phycomyces Blakesleeanus* for over 6 years and have never observed a change which permitted it to grow in the absence of thiamin. Williams (22) states that strains of *Saccharomyces cereviseae* have been cultivated in his laboratory for more than 15 years without changing their characteristics. However, the demonstration that mutations involving changes in the ability to synthesize growth substances do occur is of importance in the use of microorganisms for bio-assays, the study of the growth-substance deficiencies of microorganisms, and the investigation of the adaptive production of enzymes.

The development from the biotin-deficient (V) strain of *Fusarium avenaceum* of mutations which were autotrophic for that growth substance raises the question as to whether the (M) and (N) strains which also were biotin autotrophic may not have been derived by mutation from a biotin-deficient strain similar to the (V) strain. It is of interest to record that in our experiments we obtained from the (V) strain a biotin-autotrophic variant which closely resembled in general appearance, habit of growth, and spore form the (M) and (N) strains.

The presence of biotin in agar was previously reported by Allison and Hoover (1), Hawker (6), and Robbins (16). To find in some samples of agar as large an amount as reported here is somewhat surprising and emphasizes again the necessity of regarding agar as far more than a convenient means of solidifying a liquid medium.

Although the favorable effect of agar on the growth of *F. avenaceum* can be ascribed primarily to its biotin content our observations show that Difco agar is more effective than can be accounted for by its biotin content. It seems probable that this is due to the presence in agar of unidentified growth substances, since the addition of a readily available source of organic nitrogen (asparagine) or of mineral supplements to purified agar containing biotin did not improve the growth of *Fusarium*. One of the authors (16) has reported unidentified growth substances in agar which affect the development of *Phycomyces*, and these may be responsible also for that portion of the effect of agar on *Fusarium* which cannot be accounted for by the biotin in the agar.

SUMMARY

A strain of *F. avenaceum* did not grow in a mineral-sugar solution but grew in the same solution solidified with agar. The beneficial effect of the agar was primarily due to its biotin content. Some samples of agar were found to contain as much as 0.1 microgram of biotin per gram. The strain of *F. avenaceum* studied was favorably affected by 0.001 microgram of pure biotin methyl-ester, but greater effects were obtained with larger amounts of biotin. Extraction of agar with aqueous pyridine removed all or almost all of the biotin. Agar extract had a greater beneficial effect than could be accounted for by its biotin content. This may be the result of unidentified growth substances which are probably not pyridoxine, pantothenic acid, thiamin, nicotinamide, para-aminobenzoic acid, pimelic acid, i-inositol, riboflavine, glutamine, vitamin K_1, or ascorbic acid. Mutations which grew on a biotin-deficient medium were observed.

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RED-BLOTCH OF HIPPEASTRUM

THOMAS LASKARIS AND B. O. DODGE

(WITH FIVE FIGURES)[1]

As a rule species of *Hippeastrum* are rarely affected by any serious fungous disease. In March, 1941, Mr. E. Delafield of Riverdale-on-the-Hudson called our attention to a disease which was causing extensive damage to his collection of *Hippeastrum* (spring-flowering amaryllis). The plants, grown from bulbs purchased from Florida, showed various stages of a disease which affected the flower scapes, leaves, and bulb scales. A few weeks later similar symptoms were noted in a shipment of *Hippeastrum* obtained from Florida and grown at the New York Botanical Garden. Microscopic examination of diseased tissue revealed the presence of brown pycnidia of a fungus identified as *Stagonospora curtisii*, which was also readily obtained in pure culture from plantings of sterilized diseased tissue. The spores of this species are classed under the hyalophragmae, but one- and two-celled spores are often very common.

In this country *Stagonospora curtisii* has been described as a rather mild pathogen of amaryllis, forming conspicuous but rather small bright red spots which blemish and occasionally deform the leaves, scapes, and flowers of otherwise perfect plants. Leaf scorch, an important disease of *Narcissus*, is also caused by *S. curtisii*.

We were unable to find a description of a severe form of this disease on *Hippeastrum* such as has come under our observation. The symptoms which characterized the disease were the severe stunting and distortion of the flower stalks. Affected stalks did not make a normal upright growth but grew at an angle, some almost at a right angle to the main axis of the plants (fig. 1). This type of injury was found associated with cankers that had developed on only one side of the stalks. The fact that the cankered side of the young stalks is unable to grow as rapidly as the uninjured side doubtless accounts for the curvature in the affected stalks. A similar curvature of the stalk may result from injury at the point of emergence, a rough red bruise which may be confused with the canker caused by *Stagonospora*. The young flower shoots of *Hippeastrum* are very tightly compressed by the enveloping bulb scales, and, as they emerge, sand or other foreign particles caught between a stalk and the bulb scales may cause a scouring or roughening of the epidermis and the tissue beneath; the resultant unequal growth would cause the curvature and stunting shown in figure 5. It is quite probable that such injuries provide a favorable point of entry for *Stagonospora curtisii*, which may be present on the bulb scales. The fungus can attack the bulb

[1] Publication of the figures was assisted by the Lucien M. Underwood Memorial Fund.

scales and probably lives through the dormant period by this means. Some cankers are well developed by the time that the young shoot has emerged, which indicates that the fungus has been at work for a considerable period of time. The degree of the curvature of the stalk appears to be closely correlated with the size of the canker.

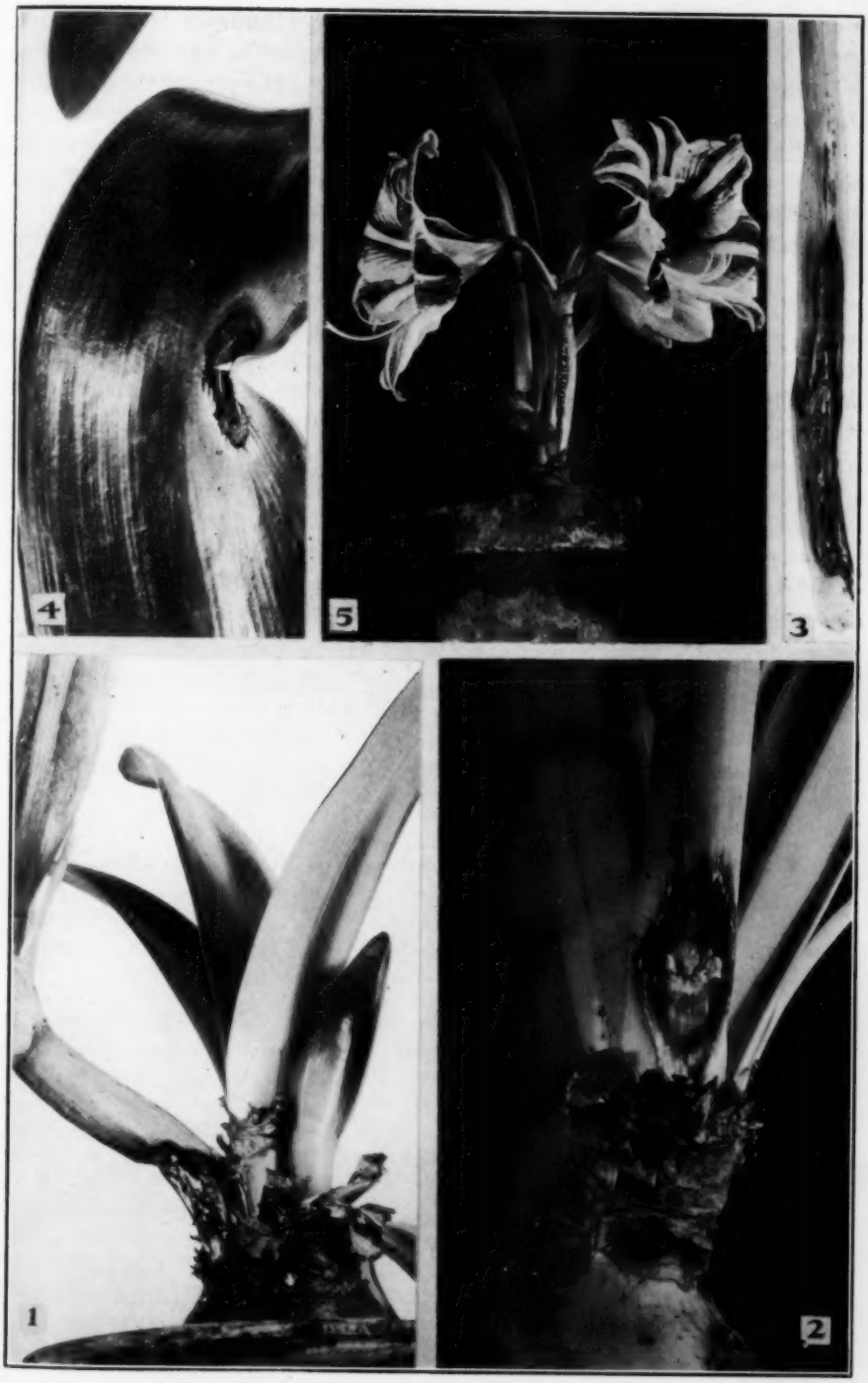
The cankers themselves are very striking in appearance. Young cankers are bright red or vermillion in color, but as they enlarge and elongate the center of the lesion becomes soft, brown and sunken. In still later stages a mat of the white or brownish gray mycelium of the causal organism develops in the center, while the borders of the canker retain the pronounced red color (fig. 1). Cankers have been observed several inches in length and from one-quarter to one-half inch in diameter (fig. 3). Elongated red spots on the leaves (fig. 4) and red streaks on the scapes, similar to those usually described as characteristic of this disease, were also observed. The flower buds borne on cankered stalks may open normally and persist in good condition for as long a period as those of healthy plants.

The pathogenicity of the fungus which was isolated has been established by inoculating amaryllis leaves with its mycelium and spores. Freshly wounded and sound leaves were inoculated with mycelium from cultures on potato-dextrose agar. Leaves of the control plants were wounded or left unwounded, and sterilized agar was placed on the injured as well as on the uninjured parts. Bell jars were placed over all the plants and removed after 48 hours. Within twenty-four hours distinct watersoaked areas were evident around the wounds. These spread rapidly, and eventually involved the greater part of the leaves. The centers of the lesions became necrotic and reddish brown, while the tissues around the necrotic regions were yellowish red in color. Similar lesions developed on leaves that were inoculated without wounding. Three weeks after inoculation the inoculated leaves were dead and dry, while no disease appeared on the controls. Repetition of these experiments with leaves cut from healthy plants and inoculated in moist chambers gave similar results.

Plants were also sprayed with a suspension of ground mycelium and pycnidia of the fungus. Bell jars were placed over inoculated and uninoculated plants and removed after 48 hours. Symptoms of infection of the

Explanation of figures 1–5.

FIG. 1. Red blotch canker at the base of a flower stalk which has been stunted and bent at right angles. Several small reddish spot infections may be seen on the flower stalk above the canker. FIG. 2. Canker at the base of flower stalk artificially infected. Mat of mycelium plainly visible on the surface. The stalk was considerably bent sidewise. FIG. 3. Canker on a flower stalk from Mr. Delafield's collection. A mass of mycelium covered some of the surface of the canker. FIG. 4. Leaf blotch which induces kinking of the leaf at the point of infection. FIG. 5. Bending and stunting of the flower stalk primarily due to mechanical injury to the epidermis and underlying tissue of the stalk as it emerges from the bulb.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inoculated plants appeared in less than a week. Hundreds of minute red spots or streaks then developed on the flowers, scape, and leaves. These enlarged during the ensuing weeks and disfigured the plants considerably. On the controls no symptom of disease appeared during the same period.

Since large cankers did not develop on any of the plants so inoculated, another experiment was made. A young flower shoot that was just emerging was injured at its base by scraping the epidermis lightly with a sterile scalpel, and a small square of an actively growing agar culture of *S. curtisii* was placed on the wounded surface. Figure 2 shows the canker that developed on this shoot after two weeks. As the canker increased in size, the stalk ceased growing in an upright direction and became bent at an angle away from the main body of the plant, thus producing the effect observed on plants naturally infected with canker.

The following genera of the Amaryllidaceae are known to be susceptible to *Stagonospora curtisii: Amaryllis, Chlidanthus, Crinum, Galanthus, Hippeastrum, Hymenocallis, Leucojum, Lycoris, Narcissus, Pancratium, Sternbergia, Sprekelia, Zephyranthes,* and *Eucharis.* Smith,[1] who studied the host range of this fungus extensively, was unable to obtain infection when he inoculated species of a number of Liliaceae, including *Hemerocallis aurantiaca.* A number of *H. fulva* seedlings inoculated by us under conditions favorable to infection failed to become diseased.

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[1] C. O. Smith. Inoculations of *Stagonospora curtisii* on the Amaryllidaceae in California. Phytopathology **25**: 262–267. 1935.

DESCRIPTIONS OF TROPICAL RUSTS—IV[1]

GEORGE B. CUMMINS

(WITH FOUR FIGURES)

Phakopsora cherimoliae (Lagerh.) Cummins, comb. nov. (*Uredo cherimoliae* Lagerh., Bull. Soc. Myc. Fr. **11**: 215. 1895; *Uredo cupulata* Ellis & Ev., Field Mus. Publ. Bot. **2**: 16. 1900; *Physopella cherimoliae* Arth., Résult. Sci. Congr. Bot. Vienne 338. 1906.)

Telia developing in the base of old uredia or usually separately in hypophyllous, subepidermal, reddish brown crusts 0.1–1 mm. in diameter and 3–6 spores in thickness, the outer layers of spores golden- or light chestnut-brown, the basal layers paler; teliospores cubical, oblong or oblong-ellipsoid, 7–13 × 13–23 μ; wall 1–2 μ thick or slightly thicker apically in the outermost spores.

On *Anona cherimolia*, GUATEMALA: Moran, Feb. 11, 1905, *W. A. Kellerman 5463*.

This rust is not uncommon, in the uredial stage, in tropical regions of the Americas from Florida to Ecuador.

ANGIOPSORA COMPRESSA Mains, Mycologia **26**: 129. 1934. (*Uredo paspalicola* P. Henn., Hedwigia **44**: 57. 1905; *Uredo stevensiana* Arth., Mycologia **7**: 326. 1915; *Puccinia compressa* Arth. & Holw.; Arthur, Proc. Am. Phil. Soc. **64**: 157. 1925; not *Puccinia compressa* Diet., Ann. Myc. **5**: 245. 1907; *P. paspalicola* Arth., Manual Rusts U. S. & Canada p. 127. 1934, in part.)

In a previous paper (Bull. Torrey Club **67**: 607. 1940) this rust was recorded for the first time in North America from Guatemala. It was stated then that it might have been previously collected but referred to *Puccinia paspalicola* (P. Henn.) Arth. (*P. tubulosa* (Pat. & Gaill.) Arth.). Subsequent examination of the rusts of *Paspalum* in the Arthur Herbarium proved that *A. compressa* is of common occurrence in tropical regions of the Americas but had been confused with various species. The species usually occurs in the uredial stage only, but I have seen telial specimens from Bolivia, Guatemala, and Puerto Rico.

As a result of these studies the above synonymy is presented. The known hosts and distribution are as follows: *Axonopus compressus:* Guatemala, Louisiana (U.S.A.), Puerto Rico; *Paspalum conjugatum:* Brazil, Cuba, Florida (U.S.A.), Guatemala, Dominican Republic, Panama, Peru, Puerto Rico, Venezuela; *P. decumbens:* Venezuela; *P. distichophyllum:* Brazil; *P. elongatum:* Bolivia; *P. fasciculatum:* Guatemala; *P. humboldtianum:* Bolivia, Guatemala, Mexico, Venezuela; *P. paniculatum:* Puerto Rico, Venezuela; *P. plicatulum:* Brazil, British Honduras, Cuba, Puerto Rico; *P. trachycauleon:* Venezuela; *P. virgatum:* Bolivia; *P.* sp.: Guatemala, Texas (U.S.A.).

Hemileia oxyanthi Cummins, sp. nov. Urediis hypophyllis, partes minores foliorum dense obtegentibus, pallide flavidis, minutissimis, per

[1] Contribution from the Department of Botany, Purdue University Agricultural Experiment Station, Lafayette, Indiana.

The third article of this series was published in Bull. Torrey Club **67**: 607–613. 1940.

stomata erumpentibus; urediosporis in apice hypharum fasciculatim erumpentium ortis, obovoideis, triangularis vel ellipsoideis, 18–23 × 24–29 μ; membranis hyalinis vel dilute flavidis, 1.5–2.5 μ cr., parte superiore moderate aculeatis, inferiore minuteque echinulatis vel fere levibus. Teliis adhuc ignotis.

On *Oxyanthus speciosus*, UGANDA: Entebbe Road, August 1940, *C. G. Hansford 2784*. TYPE specimen deposited in the Arthur Herbarium, the Herbarium of the Imperial Mycological Institute, and the Mycological Collections of the Bureau of Plant Industry, United States Department of Agriculture.

The sori usually occur in small, dense, interveinal groups but are occasionally somewhat more scattered. This specimen was made available through the courtesy of Dr. John A. Stevenson of the Bureau of Plant Industry.

Puccinia aspiliae-latifoliae Cummins, comb. nov. (*Uredo aspiliae-latifoliae* Cummins, Bull. Torrey Club **68**: 47. 1941.)

Telia not seen; teliospores in the uredia clavate or oblong-ellipsoid, rounded above, narrowed below, moderately constricted at the septum, 16–22 × (34–) 38–52 μ; wall 1–1.5 μ thick at sides, thickened to 3–5 μ at apex, yellowish or pale golden, smooth; pore apical in upper cell, next the septum in the lower cell; pedicel shorter than the spore, hyaline or yellowish, thin-walled. The teliospores germinate at once.

On *Aspilia latifolia*, SIERRA LEONE: Sefadu, Dec. 6, 1938, *F. C. Deighton 1658*.

Aside from characteristics mentioned in the description (*l.c.*) of the uredia this rust also differs from other species described on *Aspilia* because of narrower teliospores whose apical wall is only moderately thickened.

The specimen was made available through the courtesy of Dr. G. R. Bisby of the Imperial Mycological Institute.

Puccinia henryae Cummins, sp. nov. (Fig. 1.) Uredia hypophylla, sparsa, rotundata, 0.1–0.3 mm. diam., pulverulenta, obscure cinnamomeo-brunnea; urediosporae globoideae vel late ellipsoideae, 25–32 × 28–36 μ; membrana cinnamomeo-brunnea, 2.5–3 μ cr., moderate echinulata; poris germ. 2, aequatorialibus. Telia hypophylla, sparsa vel plus minusve aggregata, rotundata, 0.3–0.5 mm. diam., pulvinata, flavida; teliosporae clavatae vel oblongo-clavatae, ad apicem rotundatae, deorsum attenuatae, medio leniter constrictae, 18–25 × 40–50 μ; membrana 1.5 μ cr., ad apicem 5–8 μ cr., flavidula vel fere hyalina, levi; poro superiore apicali, inferiore juxta septum sito; pedicello persistenti, hyalino, sporam aequante. Statim germ.

On *Henrya imbricans*, GUATEMALA: Laguna (Lake Amatitlan), Dept. Amatitlan, Jan. 20, 1906, *W. A. Kellerman 5390*. TYPE deposited in the Arthur Herbarium.

This appears to be the first rust recorded on the genus *Henrya* and there is no species of *Puccinia* on other genera of the Acanthaceae to which it can be referred.

The identity of the host was recently verified by E. C. Leonard of the U. S. National Museum.

Puccinia opipara Cummins, sp. nov. (Fig. 2.) Uredia hypophylla, sparsa, ovoidea, 0.1–0.4 mm. longa, flavida; urediosporae late ellipsoideae vel ellipsoideae, 25–31 × 31–40 (–43) μ; membrana 1–1.5 μ cr., pallide flavida

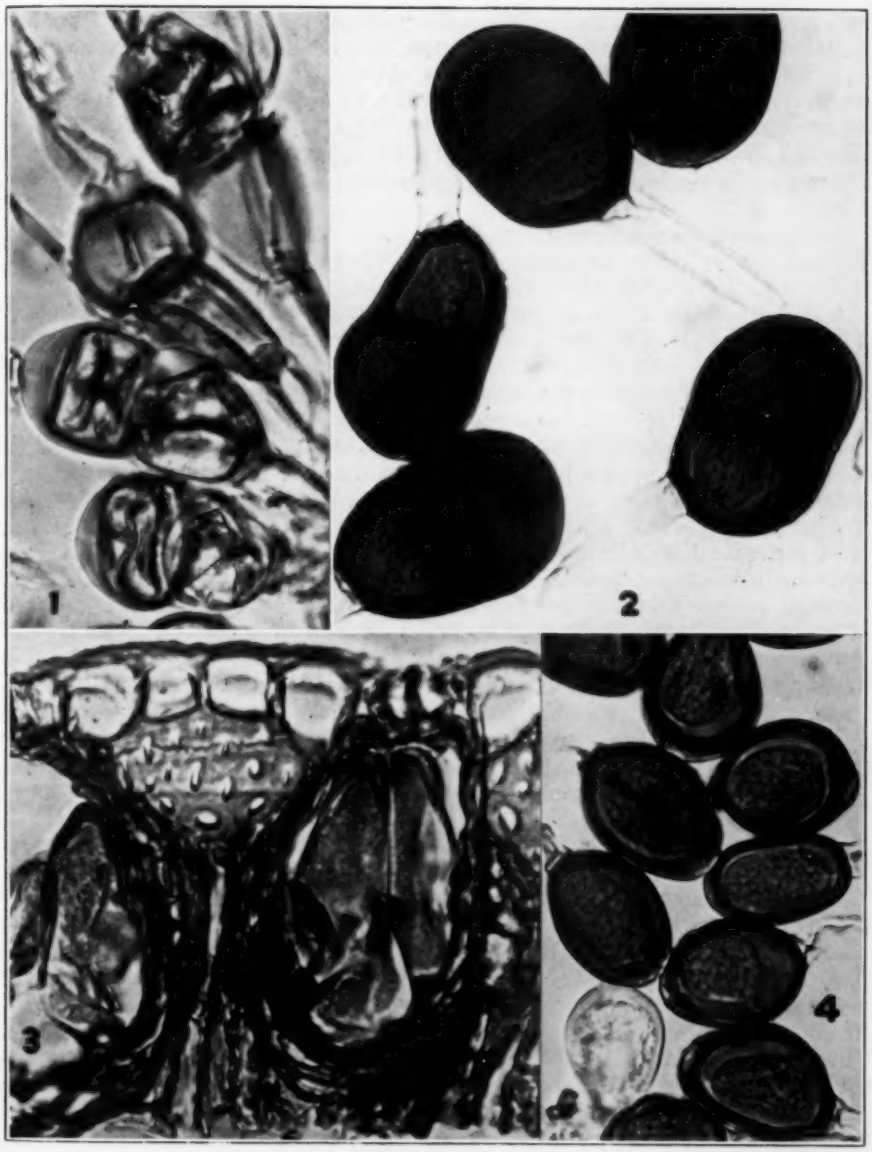

FIG. 1. Teliospores of *Puccinia henryae*, two of which have germinated and collapsed. (From type.) × 800. FIG. 2. Teliospores of *Puccinia opipara*. The wall is rather opaque and has a peculiar smoky tint. (From type.) × 800. FIG. 3. Photograph of a freehand, unstained section of the telia of *Uromyces bermudianus*, showing the substomatal position and thin peripheral layer of brownish, peridium-like hyphae. These sori are individual and not locules in a compound telium. (From type.) × 800. FIG. 4. Teliospores of *Uromyces cruckshanksiae*. The spores actually are faintly and longitudinally striate. (From type.) × 800.

vel fere hyalina, moderate echinulata; poris germ. plus minusve obscuris, 3 vel 4, aequatorialibus. Telia hypophylla, sparsa, pulvinata, rotundata vel oblonga, 0.3–0.8 mm. longa, atro-brunnea; teliosporae ellipsoideae, utrinque rotundatae vel deorsum leniter attenuatae, medio non vel vix constrictae, 23–28 (–31) × 33–44 μ; membrana 2–3 μ cr., ad apicem 4–7 μ, obscure castaneo-brunnea, levi; poro superiore apicali, inferiore juxta septum sito; pedicello sporam aequante vel longiore, pallide flavido, persistenti.

On *Oplismenus minarum*, BOLIVIA: Sorata, Apr. 17, 1920, *E.W.D.* & *Mary M. Holway 541* (TYPE); Coroico, Prov. de Nor Yungas, June 14, 1920, *E.W.D.* & *Mary M. Holway* ex *735*. Type deposited in the Arthur Herbarium.

The specimen taken as the type was originally reported by Arthur (Proc. Am. Phil. Soc. **64**: 169. 1925) as *Puccinia inclita* Arth. but was later issued in Reliquiae Holwayanae no. 82 as *Puccinia levis* (Sacc. & Bizz.) Magn. The second specimen cited was separated from Holway's no. 735 which Arthur (*l.c.* p. 176.) reported correctly as *P. levis*.

P. opipara is unlike previously described rusts on *Oplismenus*. Its teliospores have a general resemblance to those of *P. inclita* but are considerably darker in color, are more opaque and have a peculiar smoky tint. The urediospores of *P. inclita* are significantly smaller (22–26 × 24–32 μ).

Uromyces bermudianus Cummins, sp. nov. (Fig. 3.) Uredia culmicola, sparsa, ovoidea vel linearibus, 0.4–1.5 mm. longa, bullata, epidermide elevata conspicue, cinnamomeo-brunnea; urediosporae ellipsoideae vel oblongo-ellipsoideae, 16–20 (–22) × (26–) 28–35 (–37) μ; membrana pallide cinnamomeo- vel aureo-brunnea, minuteque echinulata; poris germ. 4 vel 5, aequatorialibus. Telia circum uredia denseque aggregata, subepidermalia, indehiscentibus, rotundata, 40–60 × 55–80 μ; teliosporae oblongae vel cylindraceae, 14–20 × (30–) 35–50 (–55) μ; membrana 1 μ cr., ad apicem 2–4 μ cr., aureo-brunnea vel ad basim flavidula, levi; pedicello persistenti, brevi, 5–10 μ longo, brunneolo.

On *Cyperus paniculatus*, BERMUDA: Paget Marsh, Nov. 28, 1940, *F. J. Seaver* & *J. M. Waterston 351*. TYPE deposited in the Arthur Herbarium and the Herbarium of the New York Botanical Garden.

Uromyces bermudianus is quite unlike any previously described species of *Uromyces* on *Cyperus* because of the indehiscent, substomatal, minute telia. The telia are individual, i.e. not locules separated by stromatoid paraphyses, and have only a thin peripheral layer of brown peridium-like cells. Macroscopically, however, they are greyish black and simulate multiloculate telia because they are so uniformly distributed about the uredia. Through confluence the telial spots may become continuous over areas several millimeters in length.

While there is no similar *Cyperus* rust there is a morphologically related species, *U. americanus* Speg., on *Scirpus* (see Cummins, Mycologia **27**: 611. 1935). *U. americanus* has similar sori but with the teliospores somewhat and the urediospores much longer.

Uromyces cruckshanksiae Cummins & Bonar, sp. nov. (Fig. 4.) Pycnia aequaliter sparsa, subepidermalia, amphigena, globoidea, 130–160 μ diam., paraphysata. Aecia plerumque hypophylla, ubique aequaliter denseque distributa, cupulata, 2.5–4 mm. diam., peridio lacerato vel plus minusve recurvato, cellulis peridii firme conjunctis, oblongis, cuboideis vel poly-

hedricis, 16–25 × 23–36 μ, pariete interiore verrucoso 2.5–3.5 μ cr., exteriore minuteque striato 6–8 μ cr.; aeciosporae globoideae, ellipsoideae vel oblongae, 17–22 × 19–27 μ; membrana 1–1.5 μ cr., minuteque verrucosa. Uredia non visa; urediosporae globoideae vel late ellipsoideae, 24–33 × 29–38 μ; membrana 2.5–3.5 μ cr., hyalina vel pallide flavida, moderate echinulata; poris germ. obscuris. Telia amphigena, ubique aequaliter denseque distributa, pulverulenta, castaneo-brunnea, rotundata, 0.2–0.4 mm. diam. vel confluentibus; teliosporae ellipsoideae, ovoideae vel oblongo-ellipsoideae, 17–23 × 23–30 (–34) μ; membrana 1.5–2.5 μ cr., ad apicem 3–6 μ cr., obscure cinnamomeo- vel castaneo-brunnea, minuteque longitudinaliter striata vel sublevibus; pedicello hyalino, brevi, fragili.

On *Cruckshanksia bustillosi*, CHILE: Prov. Coquimbo, Dept. Illapel, La Vega Escondida, three hours by horse east of Cuncumen, Dec. 20, 1938, *J. L. Morrison 16963;* on *C. palma*, same locality and date, *J. L. Morrison 16959* (TYPE). Type deposited in the Arthur Herbarium and the Herbarium of the University of California.

Notes made by the collector concerning the effect of this systemic rust on *C. bustillosi* are as follows: "Perennial herb. 0.1 m. fl. pink bracts; lvs. with rust are erect, the others prostrate; fls. with larger deeper colored bracts in rust-infected plants."

Cruckshanksia, a rubiaceous genus of the Chilean Andes, has not been recorded previously as a host for species of the Uredinales nor have species of *Uromyces*, with which *U. cruckshanksiae* can be confused, been described on Rubiaceae.

UREDO ROUPALAE Cumm. (Bull. Torrey Club **64**: 43. 1937) is synonymous with *Uredo mauriae* Syd. and the host is *Mauria glauca* rather than *Roupala veraguensis*.

Aecidium archibaccharidis Cummins, sp. nov. Pycnia plerumque hypophylla, subepidermalia, 90–125 μ diam., paraphysata. Aecia hypophylla, in maculis flavo-brunneis usque ad 1 cm. diam. aggregata, cupulata, 200–250 μ diam., margine recurvato; cellulis peridii firme conjunctis, flavidis, oblongis vel oblongo-ellipsoideis, 20–27 × 40–48 μ; pariete interiore moderate verrucoso 3–4 μ cr., exteriore striato 3–5 μ cr.; aeciosporae globoideae, 18–23 × 18–25 μ; membrana hyalina vel pallide flavida 1 μ cr., ad apicem 7–13 μ, laxe verrucosa, verrucis plus minusve deciduis usque ad 2.5 μ diam.

On *Archibaccharis serratifolia*, GUATEMALA: Mauchen, July 22, 1936, *J. R. Johnston 79* (TYPE), July 18, 1937, *J. R. Johnston 875*. Type deposited in the Arthur Herbarium and the Herbario de la Escuela Nacional Central de Agricultura, Chimaltenango Guatemala.

A. archibaccharidis is characterized by aeciospores with the apical wall much thickened and by the rather large, sparsely distributed and somewhat deciduous warts which form the sculpturing. The only *Baccharis* rust with similar aeciospores is *A. pachycephalum* Diet. but in it the aecia are caulicolous in position and the mycelium is systemic rather than localized.

The host was identified by P. C. Standley of the Field Museum.

Aecidium nairobianum Cummins, sp. nov. Pycnia non visa, verisimiliter nulla. Aecia plerumque hypophylla, ubique aequaliter denseque distributa, cupulata, 175–300 μ diam., margine lacerato, cellulis peridii firme conjunctis, hyalinis, globoideis, ellipsoideis vel polyhedricis, 17–25 × 25–33 μ, pariete interiore valde verrucoso 3–4 μ cr, exteriore punctato-striato 2.5–3 μ cr.;

aeciosporae variabiles late ellipsoideae, ellipsoideae vel oblongae, 13–22 × 21–33 μ; membrana 2 μ cr., hyalina, verruculosa.

On *Lippia asperifolia*, KENYA, Nairobi, June 1934, *J. McDonald*. TYPE deposited in the Arthur Herbarium and the Herbarium of the Imperial Mycological Institute.

Aecidium nairobianum differs from *A. evansii* Doidge, which has been reported on this host, and from other species on *Lippia* because of its systemic habit.

The specimen was made available through the courtesy of Dr. G. R. Bisby of the Imperial Mycological Institute.

THE ARTHUR HERBARIUM
PURDUE UNIVERSITY AGRICULTURAL EXPERIMENT STATION

STUDIES IN THE CRASSULACEAE—II. MEXICAN SEDOIDEAE COLLECTED BY E. K. BALLS IN 1938

ROBERT T. CLAUSEN

(WITH ONE FIGURE)

Through the courtesy of Dr. W. R. Maxon and Mr. C. V. Morton of the United States National Herbarium, it has been my privilege to study a small series of Crassulaceae obtained in east-central Mexico in 1938 by Mr. Edward K. Balls. The collections include specimens representing interesting variations and range extensions, also one new species of *Sedum*. In the discussion, the species are listed alphabetically.

SEDUM DENDROIDEUM Moç et Sessé. MEXICO: Cueva del Negro-Popo, 11200 ft., April 13, 1938, *E.K.B. 4192* (US 1793595). PUEBLA: Tesmalaquilla, Sierra Negra, Mt. Orizaba, 10600 ft., May 9, 1938, *E.K.B. 4470* (US 1793649). TLAXCALA: Manantiales de la Conchia, Mt. Malinche, 12400 ft., Oct. 15, 1938, *E.K.B. 5649* (US 1793885).

Inclusion of previously little known details concerning the altitudinal distribution of the species, the habitat, and flowering time renders these collections interesting and valuable. In the copious notes on the labels, the habitat is given variously as on sheer cliffs of volcanic rock in full sun, in deep shade, in rocks and steep volcanic screes in shade of cliffs or shrubs, and in deep and shaded barrancos, in moist situations, where the stems hang down from the cliffs.

The leaves of these specimens are spathulate-elliptical, blunt at apex and spurred at base, to 3.5 cm. long and 1.5 cm. wide. The largest inflorescence is 13.5 cm. long and 11 cm. wide. The flowers are yellow. In fruit, the carpels are widely spreading.

The species most closely related to *S. dendroideum* is *S. praealtum*. This differs in the pseudopetiolate leaves which are thinner, subacute, and yellow-green. The leaves of *S. dendroideum* are thick and frequently suffused with red along the margins.

SEDUM MORANENSE H.B.K. HIDALGO: Pena Baron, Parque Nacional de Miguel, 11500 ft., Aug. 11, 1938, *E.K.B. 5234* (US 1793796). VERA CRUZ: below Las Vigas, Cofre de Perote, 8600 ft., *E.K.B. 4752* (US 1793705).

This species is known to me from various places in central Mexico from southern Coahuila to Puebla. There are many collections from Hidalgo, but the specimen cited from Las Vigas is apparently the first from the State of Vera Cruz.

SEDUM NAPIFERUM Peyritsch. MEXICO: Mt. Ixtaccihuatl, 13500 ft., July 30, 1939, *E.K.B. 5178* (US 1793790).

This stonecrop has previously been collected only in the vicinity of Toluca. On Ixtaccihuatl the habitat is on rocks and open sandy slopes in moist places where water seeps through the ground. The plants are annuals, 0.5–3 cm. high. The rootstocks are tuberous-fusiform, to 1.5 cm. long, not globose as in *S. minimum* which may be only a phase of the same species. The fresh flowers are white with red centers, but in the dried condition they are mostly yellowish-white, rarely red.

Sedum obcordatum Clausen, sp. nov. Figure 1. Planta (Sectio *Dendrosedum*) glabra, lignosa, caulibus tortuosis, formans tegetes implicatas ad 60 dm. latas; folia obcordata, late rotunda, truncata vel emarginata, cuneata et sessilia, calcarata, ad 2.8 cm. longa; floris in cymis densis; bracteae florales cinereo-virides, similes foliis; sepalae erectae, lineari-oblongae, obtusae, flavae, ad 1.4 cm. longae; petalae erectae, anguste oblongae-lanceolatae, flavae, ad 1.2 cm. longae.

Plant glabrous, woody, with tortuous stems to 16 cm. or more high, forming tangled mats and tufts to 60 dm. across; leaves alternate, succulent, bluish-gray, obcordate, to 2.8 cm. long and 2.3 cm. broad, broadly rounded, truncate or emarginate, cuneate and sessile, spurred, mostly crowded towards the ends of the stems; inflorescence a dense cyme with large oblong

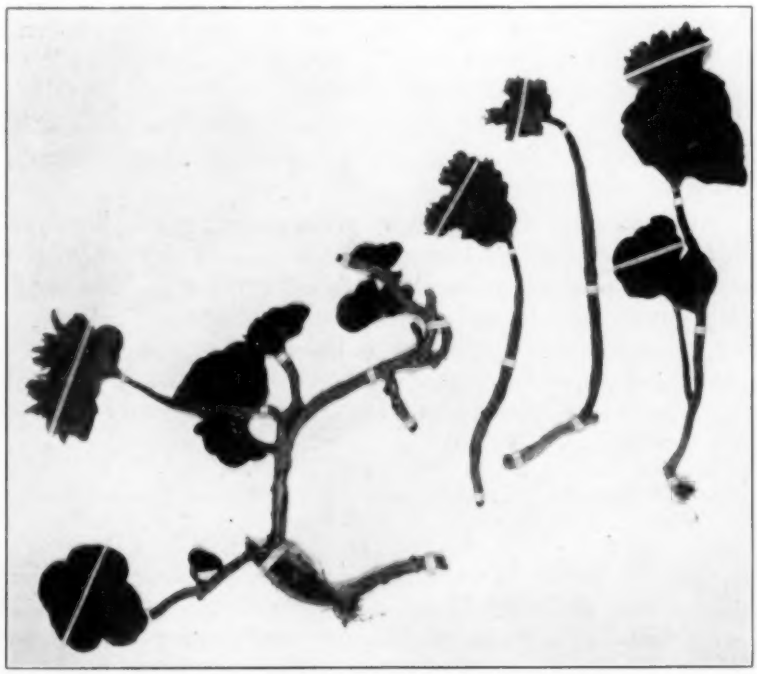

FIG. 1. Type specimens of *Sedum obcordatum* Clausen. Sheet is in the U. S. National Herbarium, no. 1793671. ×4/5.

to linear-lanceolate gray-green bracts subtended below by the proximate foliage leaves; sepals erect, linear-oblong, obtuse, unequal, to 1.4 cm. long, yellow; petals erect, narrowly oblong-lanceolate, submucronate, to 1.2 cm. long, yellow; stamens to 1 cm. long; carpels 9 mm. long, widely spreading in fruit; nectar scales transversely linear, to 1.5 mm. wide and 0.2 mm. long. Hanging from crevices in volcanic cliffs, 12500 ft., Cofre de Perote, State of Vera Cruz, May 24, 1938, *E.K.B. 4600* (TYPE, US 1793671).

This striking plant does not seem referable to any previously described species of Crassulaceae. Although it is here designated as a member of the genus *Sedum*, it in some respects resembles *Pachyphytum* and is thus intermediate between the two genera. Were a large suite of speciments and further data available, there might be basis for founding a new genus. The large floral bracts and the erect sepals and petals are unusual in *Sedum*. Yet the sepals and petals are free to the base and the petals are not appendaged as in *Pachyphytum*. Until further material and information are available, this species seems best placed in the section *Dendrosedum* of *Sedum*, which includes a group of species which are shrubs or subshrubs with yellow flowers and broad, fleshy leaves.

SEDUM OXYPETALUM H.B.K. FEDERAL DISTRICT: Camino de Toluca, 8000 ft., Feb. 10, 1938, *E.K.B. 5590* (US 1793871).

The habitat is indicated as on edges of a steep barranco, among shrubs and herbage. Although the petals are usually described as purplish red, the collector's notes give the color as white or pinkish. *Sedum conzattii* Rose, with puberulent stem and white or purple flowers, is close to this species.

VILLADIA BATESII (Hemsl.) Baehni & Macbride. FEDERAL DISTRICT: Pedregal, 7000 ft., Oct. 4, 1938, *E.K.B. 5595* (US 1793872).

Like Fröderström (1935), I find that the flowers of this species are larger than was indicated by Rose (1905). In the specimen at hand, the corolla is 5–6 mm. long and the calyx 4–5 mm. The habitat is given as sunny exposures in hot crevices in old lava.

Villadia goldmanii (Rose) Clausen, comb. nov. (*Altamiranoa goldmani* Rose, Bull. N. Y. Bot. Gard. **3**: 32. 1903). TLAXCALA: Manantiales de la Concha, Mt. Malinche, 12400 ft., Oct. 15, 1938, *E.K.B. 5650* (US 1793886).

To be consistent with the ideas expressed in my recent paper (1940), we must transfer this species to *Villadia*. One of its most striking characters is the tendency to bear scattered rosettes of leaves on the stems. According to the collector's notes, the flowers are pale pink, but Rose described them as pale yellow, tinged with red, orange-colored when dry. The collection cited is from steep sandy volcanic screes.

SUMMARY

Sedum obcordatum (Section *Dendrosedum*) is a new species from the State of Vera Cruz. *Villadia goldmanii* is a new name for the plant known until now as *Altamiranoa goldmani*. Descriptive, habitat and distributional notes are provided for *Sedum dendroideum*, *S. moranense*, *S. napiferum*, *S. oxypetalum* and *Villadia batesii*.

Bailey Hortorium
Cornell University
Ithaca, New York

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NORTH AMERICAN RANUNCULI—II[1]

LYMAN BENSON

8. RANUNCULUS RECURVATUS Poir. in Lam. Encyc. Meth. **6**: 125. 1804. *Ranunculus recurvatus* var. *adpressipilis* Weatherby, Rhodora **31**: 164. 1929. *Ranunculus recurvatus* f. *laevicaulis* Harger ex Weatherby, Rhodora **31**: 164. 1929. *Ranunculus recurvatus* var. *fontinalis* Peattie, Jour. Elisha Mitchell Soc. **44**: 205. 1929. *Ranunculus recurvatus* f. *Hargeri* Weatherby ex Peattie, Jour. Elisha Mitchell Soc. **46**: 155. 1931, as syn. (incorrectly ascribed to Weatherby).

Woods and bottom-land thickets at low elevations; Ottawa, Ontario, and Minneapolis, Minnesota, and eastward to Newfoundland and southward to Oklahoma (Dripping Springs), Louisiana, and Georgia. Coniferous and deciduous forest belts. Late April to June.

Type collections: (1) *R. recurvatus*, "Cette plante croîte en Amérique, dans les environs de New York . . . in herb. Lamarck." (2) Var. *adpressipilis*, "VIRGINIA: Hungry Hollow, alt. 2200 ft., northeast of Marion, Smyth Co., May 24, 1892, Small, TYPE in Grey Herb." This form is remarkable for appressed hairs, which are unicellular. (3) F. *laevicaulis*, "MASSACHUSETTS: by brook, Whately, May 17, 1913, Fernald & Harger, Type in hb. N. E. B. C. (also a mixed sheet in Herb. Gray). (4) Var. *fontinalis*, "Type specimens: In a waterfall, April 9, 1919 (Day). In a waterfall, Melrose Mt., April 3, 1929 (PT. 068). Pearson's Falls, in the water, May 11, 1926 (PT. no. 2188)." Polk County, North Carolina and adjacent South Carolina. (PT. = Peattie.)

9. RANUNCULUS BONGARDI Greene, Erythea **3**: 54. 1895. *Ranunculus occidentalis* Nutt. var. *parviflorus* Torr. Bot. Wilkes Exped. **17**: 214. 1874. *Ranunculus occidentalis* var. *Lyallii* A. Gray, Proc. Am. Acad. **21**: 373. 1886. *Ranunculus tenellus* Nutt. var. *Lyallii* Rob. in A. Gray, Syn. Fl. N. Am. **1**: 33. 1895. *Ranunculus Greenei* Howell, Fl. N. W. Am. **1**: 18. 1897. *Ranunculus Lyallii* Rydb. Mem. N. Y. Bot. Gard. **1**: 166. 1900. *Ranunculus Bongardi Greenei* Piper, Contr. U. S. Nat. Herb. **11**: 275. 1906.

Hispid perennials; stems 2–6 mm. in diameter, hispid with the hairs commonly reddish-brown; radical leaf blades 3–9 cm. long, 4–14 cm. broad, 3-parted, the parts lobed and acutely toothed, appressed-hispidulous; petioles densely hispid, the hairs usually reddish-brown; cauline leaves similar to and usually smaller than the radical; achenes 8–20 or 25 in a globose-hemispherical cluster 3–4 mm. long by 4 mm. in diameter, each achene obovate, 1.8 mm. long, 1.3–1.5 mm. dorsoventrally, 0.8 mm. laterally,

[1] The first article in this series, Bull. Torrey Club **68**: 157–172. 1941, includes a key to the species of the section *Chrysanthe* of the subgenus *Euranunculus* and discussion of the first seven species, *Ranunculus acris*, *R. acriformis*, *R. repens*, *R. bulbosus*, *R. occidentalis*, *R. californicus* and *R. canus*. The present delimitation of subgenera and sections was published in an article entitled "The North American subdivisions of *Ranunculus*," Am. Jour. Bot. **27** (9): 799–807. 1940.

smooth or with a trace of reticulation, hispid, the achene beak slender but broad at the base, 2 mm. long, recurved, hooked at the tip.

Shaded and rather moist places 50–1,000 meters or inland up to 2,000 meters elevation; Pacific slope from Kodiak, Alaska, to Humboldt County, California; occasional in the interior as follows: Kootenai District, British Columbia; northeastern and southeastern corners of Washington; Deschutes River and northeastern Oregon; Warner Valley, Mohawk Valley, and the San Bernardino Mountains, California; Idaho, western Montana, and northwestern Wyoming; North Park, Colorado. Northwestern coniferous and yellow pine forests. Late April to July.

The form occasional in the Rocky Mountains and in the Blue Mountains of Washington and Oregon and the Wallowa Mountains of Oregon has hair around only the margin of the achene, and it is perhaps more closely allied to *Ranunculus Bongardi* var. *tenellus*.

Type collections: (1) Var. *parviflorus*, "Plains between Puget Sound and the Cascade Mountains." *Wilkes Expedition*. (2) Var. *Lyallii*, "Lyall's specimens are from Pend Oreille River, in Idaho." (3) *R. Bongardi*, "**Ranunculus Bongardi.** *R. recurvatus*, Bong. Veg. Sitch mainly, not of Poiret." (4) *R. Greenei*. "*R. occidentalis* var. *Lyallii Gray, Proc. Am. Acad. xxi, 373.* . . . Common in open Fir forest, Oregon to British Columbia." Since no specimen is mentioned, *R. Greenei* is considered to have been proposed as a new name for Gray's var. *Lyallii* and to be based upon the same type.

Significant specimens: cf. L. Benson, Am. Jour. Bot. **23**: 30–31. 1936.

9A. Ranunculus Bongardi var. tenellus (Nutt.) Greene, Erythea **3**: 54. 1 Ap 1895. *Ranunculus tenellus* Nutt. in Torr. & Gray, Fl. N. Am. **1**: 23. 1838, not Viviani in 1831. *Ranunculus Nelsonii* (DC.) A. Gray, var. *tenellus* A. Gray, Proc. Am. Acad. **8**: 374. 1872. *Ranunculus occidentalis* Nutt. var. *tenellus* A. Gray, Proc. Am. Acad. **21**: 373. 1886. *Ranunculus Nelsonii glabriusculus* Holzinger, Contr. U. S. Nat. Herb. **3**: 210. 23 N 1895. *Ranunculus Douglasii* Howell, Fl. N. W. Am. **1**: 18. March, 1897. *Ranunculus arcuatus* Heller, Bull. Torrey Club **24**: 319. 29 Je 1897. *Ranunculus Bongardi* var. *Douglasii* Davis, Minn. Bot. Studies **2**: 479. 1900.

Sparsely hirsute or glabrous terrestrial annuals or sometimes perhaps perennials; stems 2–4 or 5 mm. in diameter, glabrous or somewhat hirsute with soft white hairs; radical leaf blades 2–5 or rarely 7.5 cm. long, 2–7 or rarely 13 cm. broad, 3-parted and the parts lobed, the ultimate lobes obtuse or sometimes acute, glabrous; petioles glabrous or somewhat hirsute; cauline leaves usually larger than the radical; achenes 5 or 12–30 in a globose-ovoid or hemispherical cluster 4–5 mm. long by 4–5 mm. in diameter, each achene discoid, obovate, or elliptic, 2–2.5 mm. long, 1.7–1.9 mm. dorsoventrally, 0.8 mm. laterally, smooth or minutely reticulate, glabrous, the achene beak 1–1.5 mm. long, 0.3–0.5 mm. broad at the base, recurved and hooked at the tip.

Moist shaded ground at 50–1,000 meters elevation near the coast and up to 2,100 meters in the interior; southern coastal Alaska; Vancouver Island,

British Columbia; Puget Sound region; western Oregon to Humboldt County and the Sierra Nevada and San Bernardino Mountains, California; most common in the mountainous parts of the Great Basin in eastern Washington, Oregon, and California and in Idaho and western Montana; Yellowstone National Park and the Bighorn Mountains, Wyoming. On the Pacific slope the plant is localized mostly where rivers cut through the Cascade Mountains from the Great Basin. Northwestern coniferous and (mostly) yellow pine forest. May to July.

At Snoqualmie Falls, Washington on May 4, 1929, this species was found to be in flower and young fruit (*L. Benson 1228, 1229, 1230*) while *R. Bongardi* growing with it was not yet in flower (*L. Benson 1231*). Where the species and variety grow together, the characters of *R. Bongardi* and var. *tenellus* occur sometimes in various recombinations. However, the mostly coastal *R. Bongardi* and the largely interior var. *tenellus* are significantly different in several characters, and, despite intergradation, some might maintain them as separate species. The presence or absence of hair on the fruit has been adopted as an arbitrary criterion for segregating intermediate plants having characters of both the typical species and the variety.

Type collections: (1) *R. tenellus,* "Shady woods of the Oregon [Columbia] and Wahlamet [Willamette] Rivers, *Nuttall!*" (2) *R. Nelsonii glabriusculus,* "*R. nelsonii tenellus* Gray, Proc. Amer. Acad. viii, 374 (1872); *R. tenellus* Nutt.; Torr. & Gray Fl. i, 23. (1838), not Viviani. . . . Rich bottoms near Lapwai Agency, Nez Perces County, Idaho; May 5 (No. 126). Also on Clearwater River near mouth of Big Potlatch River and in the valley of the Little Potlatch." Apparently the name is merely a new one for *R. Nelsonii* var. *tenellus,* and none of the three collections listed (made by J. H. Sandberg and assistants in 1892) is intended as a type. (3) *R. Douglasii,* "*R tenellus Nutt. T. & G. Fl. i. 23. not Viviani.* . . . Common in moist places and river bottoms, California to Brit. Columbia." Since no specimen is mentioned, *R. Douglasii* is believed to have been proposed as a new name for *R. tenellus* Nutt. and to be based upon the same type. (4) *R. arcuatus.* Nomen novum for *R. tenellus* Nutt.

Significant specimens: cf. L. Benson, Am. Jour. Bot. **23**: 31. 1936.

9B. RANUNCULUS BONGARDII var. **Earlei** (Greene) L. Benson, comb. nov. *Ranunculus Earlei* Greene, Pittonia **4**: 15. 1899.

Perennial; basal leaves larger than the cauline, or only the bracts present, pilose with hairs 1–1.3 mm. long; petals obovate, 3.5–7 mm. long by 2–3 mm. broad; achenes 15–40 in a subglobose or ovoid head, each 3 mm. long, the beak 1 mm. long, 0.5–0.7 mm. broad at the base; receptacle in fruit 3 mm. long, ovoid; otherwise like var. *tenellus.*

Along streams at 2,250–2,550 meters elevation; near Mt. Hesperus and Mancos and in Delta and San Miguel Counties, Colorado. Yellow pine forest. June.

Related to *R. acriformis* A. Gray.

Type collection: "Along the Mancos River and other streams, June 22, and 28, Baker, Earle, and Tracy (38, 39, 187)." *Baker, Earle, & Tracy 38,* collected along "Mancos River Bottoms, 7,000 ft." is designated as a LECTOTYPE. It is *HGr. 2565.*

Significant specimens: COLORADO: Mancos River, *Baker, Earle, & Tracy 38,* HGr, B, US, NY; *39,* HGr, B, NY; Bob Creek, West La Plata Mountains, west of Mt. Hesperus, *Baker, Earle, & Tracy 187,* HGr, NY, GH; Leroux Creek, Delta County, *Cowen 29* in 1892, NY; Umcompahgre Plateau, West Fork of Dry Creek, *Tidestrom 1556,* US; Trout Lake, San Miguel County, *Payson & Payson 4148,* GH.

10. RANUNCULUS MACOUNII Britt. Trans. N. Y. Acad. Sci. **12**: 3. 1892. *Ranunculus hispidus* Michx. var. *oreganus* A. Gray, Proc. Am. Acad. **21**: 376. 1886. *Ranunculus oreganus* Howell, Fl. N. W. Am. **1**: 19. 1897. *Ranunculus Macounii* var. *oreganus* Davis, Minn. Bot. Studies **2**: 469. 1900. *Ranunculus rudis* Greene, Ottawa Nat. **16**: 33. 1902. *Ranunculus oreganus Macounii* Piper, Contr. U. S. Nat. Herb. **11**: 276. 1906. *Ranunculus rivularis* Rydb. Bull. Torrey Club **39**: 319. 1912.

Muddy ground of marshes, lake and stream borders, and ditches from sea level on the north coast to 2,400 meters elevation in the Rocky Mountains; Siberia; Alaska to Labrador (Lake Melville) and Newfoundland and southward to California (Goose Lake), eastern Arizona, montane New Mexico, Iowa, and Michigan (Isle Royle). Late May to August.

A geographically isolated glabrous form (var. *oreganus*) occurs at Pullman, Washington, along the lower Columbia River, and in Alaska and Siberia. In the northern part of the range of *R. Macounii* adventitious rooting is not found or is at least uncommon. Rydberg, *loc. cit.,* has proposed segregation of the rooting form as a separate species. This certainly is not justified, although it is possible that the plant might be recognized as a variety. As far as it is possible to judge from herbarium sheets, the nonrooting form occurs mostly from Washington to Colorado and northward and the rooting form occurs in those states and southward. However, adventitious roots occur on even some plants from Newfoundland. Herbarium specimens are inadequate for a clear segregation of the two forms, and other characteristics do not seem to be clearly associated with rooting and nonrooting. Much of the southwestern material is perennial, and many of the erect plants from especially the northern part of the range are apparently annual. One specimen with flowers and young fruit (Round Lake, Mackenzie Basin, northeastern Alberta, *Raup 2361,* NY.) shows the pair of cotyledons still attached to the diminutive plant in anthesis. A larger plant (*Raup 2360,* NY.) from Lake Mamawi, Mackenzie Basin is almost undoubtedly annual also, but of normal size, and a normal-sized duplicate of *Raup 2361* is in the Gray Herbarium.

The plant keyed out near *Ranunculus recurvatus* is *H. M. Raup 2367* from a slough margin at Wood Buffalo Park, Mackenzie Basin. This plant

and some other specimens from the vicinity may be confused with *R. recurvatus* and *R. Bongardi*, but apparently they are reduced forms of *R. Macounii*.

Type collections: (1.) Var. *oreganus*, based upon "*R. nitidus*, in part, Hook. Fl. i. 20—Shady and wet grounds, Oregon, on the Columbia, to Fraser River." According to Hooker, *l. c.*, "Abundant on the lower fertile plains of the Columbia, where it attains a height of 1½ to 2½ feet, extending into the mountain valleys, where it is of humbler growth. *Douglas.*" (2.) *R. Macounii*, based upon *R. hispidus* Hook. Fl. Bor. Amer. **1**: 19. 1829, not Michx. in 1803. "Banks of rivers from Canada to near the mouth of the Mackenzie River lat. 67°; and from the shores of Hudson's Bay to the Pacific. *Dr. Richardson. Drummond. Scouler. Douglas.*" (3.) *R. rudis*, "Discovered in a wet meadow in Devil's Garden, northern California, (Plumas or Lassen County) June, 1895, by *Mrs. R. M. Austin.*" The type is *HGr. 2796–7*. (4.) *R. rivularis*, "NEVADA: Huntington Valley, August, 1868, *S. Watson 27* (type, in herb. Columbia University)." New York Botanical Garden.

11. RANUNCULUS PENSYLVANICUS L. f. Suppl. 272. 1781.

Wet ground of woods, meadows, and bottom lands at low or moderate elevations; China; Juneau, Alaska to Puget Sound, Yakima, and northeastern Washington and eastward across Canada and the two northernmost tiers of states to Newfoundland and New Jersey; occasional southward in the Rocky Mountains as far as northeastern Arizona and New Mexico (Rio Arriba County and the Datil Mountains).

The plants from Arizona and New Mexico have shorter fruiting receptacles and so also have specimens from Itasca Park, Minnesota (*Mayer 318, NY*).

Type collection: "*Habitat* in Pensylvania."

Significant specimens: ALASKA: Juneau, *Anderson 461*, NY. WASHINGTON: cf. L. Benson, Am. Jour. Bot. **23**: 32. 1936. MONTANA: East Gallatin swamps, *Rydberg 478*, NY; Big Fork, *B. T. Butler 7002*, NY; *7010*, NY; Columbia Falls, *R. S. Williams* in 1892, NY. IDAHO: Falks Store, Canyon County, *Macbride 316*, NY; Rathdrum, Kootenai County, *Sandberg, MacDougal, and Heller 732*, NY. ARIZONA: Ryan Ranch, East Fork of White River, *Harrison 4868*, Sac.

For the spelling of the specific name, cf. Fern. Rhodora **42**: 94–5. 1940.

12. RANUNCULUS MACRANTHUS Scheele, Linnaea **21**: 585. 1848. *Ranunculus repens* L. var. *macranthus* A. Gray, Bost. Jour. Nat. Hist. **6**: 141. 1845.

Moist meadows at about 2,000 meters elevation in Eastern Arizona from Navajo County to the Santa Rita and Huachuca Mountains and at lower elevations in Western and Southern Texas; Mexico. Yellow pine and southern hardwoods forests; Gulf grassland. June and July in Arizona and March and April in Texas.

Type collection: "Prope Neubraunfels leg. Römer. Aprili." Texas.

13. RANUNCULUS ORTHORHYNCHUS Hook. Fl. Bor. Amer. **1**: 21. *pl. 9.* 1829. *Ranunculus ornithorhynchus* Walp. Rep. **1**: 43. 1842, error in spelling. *Ranunculus orthorhynchus* "form" *stenophyllus* A. Gray, Proc. Am. Acad. **21**: 377. 1886.

Stems 1.5–5 dm. long and 1.5–3 or 4 mm. in diameter, fistulous, hirsute, the hairs ascending and tending to be appressed; radical leaf blades pinnate with 3–7 leaflets, the leaflets alternate or opposite, again twice-forked or -lobed, the divisions all (typically) linear or else cuneate, ultimate lobes acute, appressed-pubescent or nearly glabrous; petioles 3–12 cm. long; sepals 5, greenish-yellow, pilose dorsally; petals yellow or (in the Northwest) often red dorsally, 8– or 10–19 mm. long, 4–7 mm. broad; achenes 12–20, each achene elliptic, 3 mm. long, 2.3 mm. dorsoventrally, 0.5 mm. laterally, margin prominently bevelled and slightly keeled, the achene beak slender, about 4 mm. long, straight; receptacle nearly cylindrical, 1–2 mm. long in flower and 2–3 mm. long in fruit, hispidulous.

Meadowland at low elevations along the northwest coast and at 1,500–2,000 meters elevation southward; Pacific slope from Vancouver Island to western Oregon; Goose Lake, middle north Coast Ranges, and central Sierra Nevada, California; one fragment from Yellowstone National Park. Northwestern coniferous and yellow pine forests. May and June.

Where *Ranunculus orthorhynchus* crosses the range of var. *platyphyllus* in southwestern Oregon and adjacent California, intergrades are abundant. The Sierra Nevada plants are also intermediates, and their assignment to *R. orthorhynchus* is rather arbitrary.

Type collections: (1) *R. orthorhynchus,* "Not unfrequent on the low points of land near rivers, in North-West America. *Douglas.*" (2) "Form" *stenophyllus,* "The typical form of *R. orthorhynchus,* STENOPHYLLUS, has all the leaves somewhat bipinnately dissected into segments a line or less in width, or some radical ones simply divided into cuneate or obovate 2–3-lobed or toothed segments or leaflets." The type must be identical with that of *R. orthorhynchus.* Gray cited no specimens or localities for the "form."

13A. RANUNCULUS ORTHORHYNCHUS var. PLATYPHYLLUS A. Gray, Proc. Am. Acad. **21**: 377. 1886. *Ranunculus maximus* Greene, Bull. Torrey Club **14**: 118. 1887. *Ranunculus orthorhynchus* var. *maximus* Jepson. Fl. W. Mid. Calif. 200. 1901. *Ranunculus politus* Greene, Pittonia **5**: 196. 1903. *Ranunculus platyphyllus* A. Nels. Bot. Gaz. **42**: 52. 1906. *Ranunculus platyphyllus* Piper, Contr. U. S. Nat. Herb. **11**: 276. 1906.

Stems 6–12 dm. long, often 7–9 mm. in diameter, hirsute, sometimes rather densely so; basal leaves pinnate with 5–7 leaflets; sepals hirsute dorsally; petals broader and shorter than in the typical species, rarely red dorsally (southern Oregon); achenes 20–35, achene beak 2.5–3.5 mm. long and less rigid than in the typical species, sometimes very nearly hooked at the apex; receptacle elongated from 2–3 mm. long in anthesis to 5–9 mm. long (and expanded to 2–3 mm. in diameter) in fruit.

Meadows from sea level on the coast to 2,200 meters elevation in the interior; California along the coast from Del Norte County to Berkeley;

upper Sacramento Valley and at Stockton; mountains of the Great Basin from the southern edge of British Columbia to eastern Oregon, Nevada (Gold Creek), Utah (Wasatch and Uintah Mountains), Wyoming (West De Lacy Creek and Teton Pass), and Glacier National Park, Montana (San Marias Pass, *Somes 90*). Northwestern coniferous and western pine forests and Pacific grassland. June and July.

Apparently originally introduced into California by the Klamath River, which intersects the range of *Ranunculus orthorhynchus*. Where the ranges of the species and variety overlap, hybridization is frequent, and segregation is difficult.

Type collections: (1) Var. *platyphyllus*, "*R. macranthus*, Watson, Bot. King Exped. and Bot. Calif., not Scheele.—In wet places, Wahsatch Mountains and Idaho to E. Oregon, and California, south to Marin Co." The following is quoted from Watson, Rept. U. S. Geol. Expl. 40th Par. **5**: 9. 1871, "RANUNCULUS MACRANTHUS, Scheele. . . . Texas, California, (4,729 Bolander), and Oregon, (Lyall.) Streambanks in the Wahsatch and Uintas; 5–8,000 feet altitude; June, July. (28)." The specimen in the Gray Herbarium from the Wasatch Mountains, *S. Watson 28*, collected in 1869, is designated as a lectotype. (2) *R. maximus*. "*R. macranthus*, Brew. & Wats., Bot. Cal. i. 8, not of Scheele; *R. orthorhynchus*, var. *platyphyllus*, Gray, Proc. Am. Acad. xxi. 377, as to the Californian plant at least." Brewer and Watson refer the reader to "Watson, Bot. King 9," and they state further, "Moist soils from Oregon to Nevada and Texas. In this state [California] near the coast." The reference to *R. orthorhynchus* var. *platyphyllus* includes specifically only the "Californian plant." Since both var. *platyphyllus* and *R. macranthus* of the Botany of California were based upon Watson's previous publication (Rept. U. S. Geol. Expl. 40th. Par. 5: 9. 1871, as cited above), the single California specimen mentioned there is designated as a lectotype. It is *Bolander 4729* from Long Valley Mendocino County. LECTOTYPE in the Gray Herbarium.

13B. RANUNCULUS ORTHORHYNCHUS var. HALLII Jepson, Fl. Calif. **1**: 542. 1922.

Hair of stems and petioles spreading; leaf divisions often as broad as long, shallowly and obtusely lobed; achenes 4–17, usually few, the marginal keel of each more distinctly carried into the beak than in the typical species; otherwise like the typical species.

Mountain meadows at about 2,000 meters elevation; Sierra Nevada from Yosemite National Park to Fresno County, California. Yellow pine forest. June and July.

Type collection: "Pine Ridge, Fresno Co., Hall and Chandler 236 (type)."

Significant specimens: cf. L. Benson, Am. Jour. Bot. **23**: 31–32. 1936.

13C. RANUNCULUS ORTHORHYNCHUS var. **alaschensis** L. Benson var. nov.

Stems 6–7 dm. long, 2–4 mm. in diameter, markedly fistulous, glabrous; basal leaves with 3 or 5 leaflets, the terminal leaflet 3-lobed and the lobes

again notched, the lateral leaflets opposite or nearly so, cuneate to obdeltoid, 15–30 mm. long, of 13–30 mm. maximum breadth, the ultimate lobes rounded and the sinuses acute, petioles 15–20 cm. long; cauline leaves more dissected than in the typical species and with acute ultimate lobes; sepals glabrous, tinged with purple; petals 5, yellow on both sides, obovate, 11–12 mm. long, 6 mm. broad; achenes 10–20, the achene body 4 mm. long, 3 mm. broad, the margins not markedly beveled or keeled, the beak straight or a little curved at the tip, 3 mm. long; receptacle but slightly enlarged in fruit, hirsute.

Herba terrestris perennis glabra; caulibus 6–7 dm. longis, 2–4 mm. diametro, glabris; foliis 3–5-foliolatis, foliolis cuneatis vel obdeltoideis, tridentatis, 30 mm. longis, 13–30 mm. latis; petiolis 15–20 cm. longis; sepalis glabris; petalis 5, luteis, obovatis, 11–12 mm. longis, 6 mm. latis; acheniis 10–20, obovatis, 4 mm. longis, 3 mm. latis; rostro filiformi, 3 mm. longo.

Moist ground at low elevations; Yes Bay and Anan Creek, south coast of Alaska. Northwestern coniferous forest. July.

Type collection: Shores of Yes Bay, Alaska, July 20, 1895, *Thos. Howell, Howell's Pacific Coast Plants No. 1603.* TYPE in the New York Botanical Garden.

Significant specimens: ALASKA: Yes Bay, *Howell 1603*, NY, HGr; *Gorman 61*, NY; Anan Creek, mainland, *Walker 761*, NY, GH.

14. RANUNCULUS BLOOMERI S. Wats. Bot. Calif. **2**: 426. 1880.

Very wet and heavy soil of meadows, sloughs, and ditches; Mendocino and Lake Counties to Santa Clara County, California. Oak woodland. March to early May.

Intergrades with *Ranunculus orthorhynchus* var. *platyphyllus* occur abundantly in western Lake County and northeastern Sonoma County.

Type collection: "In wet grounds near San Francisco, *Dr. J. G. Bloomer.* The specimens are imperfect and not yet in fruit, but indicate a very distinct species."

15. RANUNCULUS CAROLINIANUS DC. Syst. **1**: 292. 1818.

Swampy ground at low elevations near the coast; Alabama to North Carolina and Florida. River bottom forest. March to May.

Type collection: "Hab. in Carolina inferiore. *Bosc.*"

This species has been known as *R. palmatus* Ell.; cf. Fern. Rhodora **41**: 543–4. 1939.

Table 2 contrasts *Ranunculus carolinianus* with its eastern relatives, *R. hispidus* and *R. septentrionalis:*

16. RANUNCULUS SEPTENTRIONALIS Poir. in Lam. Encyc. Meth. **6**: 125. 1804. *Ranunculus lucidus* Poir. in Lam. Encyc. Meth. **6**: 113. 1804 (?). *Ranunculus tomentosus* Poir. in Lam. Encyc. Meth. **6**: 127. 1804, not Moench in 1794. *Ranunculus Belvisii* DC. Syst. **1**: 291. 1818. *Ranunculus nitidus* Muhl. Cat. Ed. 2. 56. 1818, not Walt. in 1788. *Ranunculus intermedius* Eat. Man. Bot. Ed. 3. 424. 1822, not Poir. in 1804. *Ranunculus Schlectendalii*

TABLE 2

	R. carolinianus	*R. hispidus*	*R. septentrionalis*
Petal breadth	2–3 or rarely 4 mm.	4–7 or rarely 3 or 11 cm.	4–8 or 13 cm.
Sepal habit	Reflexed	Spreading	Usually spreading
Comparative length of sepals to petals	Half	Half	Two-thirds to three-fourths
Achene number	7–15	12–30	15–30
Stoloniferous	Rarely	Never	Frequently (if not always) after flowering
Stem diameter	0.7–1.5 or 2 cm.	1.5–2.5 cm.	1.5 or 2–8 cm.
Stamen number (approximation)	20–40	40–60	40–75
Length of fruiting receptacle	2–3 mm.	4–8 mm.	4–8.5 mm.

Hook. Fl. Bor. Amer. **1**: 21. 1829. *Ranunculus septentrionalis* var. *nitidus* Chapm. Fl. S. U. S. Ed. 2, Suppl. 675. 1892. *Ranunculus octopetalus* Greene, Ottawa Nat. **16**: 33. 1902. *Ranunculus caricetorum* Greene, Pittonia **5**: 194. 1903. *Ranunculus sicaeformis* Mack & Bush apud Mack., Torreya **6**: 123. 1906. *Ranunculus sicaefolius* Mack. & Bush ex Rydb. Fl. Prairies & Plains of Central No. Amer. 342. 1932, *nomen nudum*, apparently intended for *R. sicaeformis*. *Ranunculus septentrionalis* var. *caricetorum* Fern. Rhodora **38**: 177. 1936.

Subglabrous to densely hispid; stems erect and 2–6 dm. high or (in summer) at least sometimes stoloniferous; radical leaf blades compound or occasionally simple and 3-lobed, broadly ovate-cordate in outline, 3–14 cm. long, 5–20 cm. broad, the leaflets 3-lobed, -cleft, or -parted and again toothed or lobed, the blade proximally truncate or cordate and distally angular, subglabrous to appressed-hispidulose; sepals spreading or (in some Middle-Western plants) reflexed; achenes about 15–30, each achene obovate, 3–3.5 mm. long, 2–2.5 mm. dorso-ventrally, 0.7 mm. laterally, the achene beak stout at the base, slender above, 2–3 mm. long, straight; receptacle 4–8.5 mm. long in fruit.

Marshy ground at mostly low elevations; Eastern South Dakota and Nebraska to James Bay and to Battle Harbor, Labrador and southward to Missouri, Kentucky (*Short* in 1840, N.Y.), West Virginia, and the Virginia Coastal Plain; Fulton, Arkansas and Austin, Texas. April to early June (July or August northward).

Reported by Fernald (Rhodora **22**: 31. 1920) to produce long stolons very soon after the beginning of flowering. The writer has collected this species only once (in Pennsylvania in June, 1935), and in this case field observation revealed an abundance of stolons. Most herbarium specimens do not show stolons. The densely retrorsely-hispid form occurring here and there in the Middle West and at Cumberland, Maryland (*Shriver* in 1894, NY) is var. *caricetorum* (Greene) Fern.

Type collections: (1) *R. septentrionalis,* "Cette plante croît dans l'Amerique septentrionale . . . in herb. Lamarck." (2) *R. lucidus,* "Cette plante est cultivée au Jardin des Plantes de Paris. On soupçonne originaire du Levant." (3) *R. tomentosus,* "Cette plante a été recueillie par M. Bosc dans la Haute-Caroline." (4) *R. Belvisii,* "Hab. in America boreali. *Pal. de Beauvois.*" (5) *R. nitidus,* "Car." Carolina. Type in the herbarium of Muhlenberg. (6) *R. intermedius,* "Found on the banks of the Hudson near Albany, by Mr. J. G. Tracy." (7) *R. Schlectendalii,* "R. fascicularis, *Schlect. Animadv.* Sect. 2, p. 30. t. 2. . . . Eastern declivity of the Rocky Mountains, between lat. 52° and 55°, in rich soils: plentiful. *Drummond.*— This plant agrees in every particular, as far as I can judge without fruit, with the description and figure above quoted of Schlectendal's *R. fascicularis,* except that his figure represents a slenderer plant, and one of the leaves has the middle lobe petioled, and the calyx reflexed. . . ." Asa Gray, Proc. Am. Acad. **21**: 372–3, 376. 1886, designated Schlectendal's specimen as a LECTOTYPE for *R. Schlectendalii.* (8) *R. octopetalus,* "Knox Co., Tennessee, 10 June, 1893, T. H. Kearney." The TYPE specimen is *HGr. 2739,* and the specimen from marshes near Fountain City, *Kearney,* June 10, 1893, *HGr. 2738,* is probably part of the same collection. (9) *R. caricetorum,* "The above diagnosis is from material of my own gathering in southern Wisconsin in 1888, and in southern Michigan in 1902." The specimen from Dodgeville, Wisconsin, collected by Greene June 20, 1898, is designated as a lectotype. It is *HGr. 2559–60–61.* (10) *R. sicaeformis,* "Collected by myself (no. 95) at Buckner, Jackson County, Missouri, on May 30, 1898 . . . in my herbarium," *K. K. Mackenzie.* The TYPE is now in the New York Botanical Garden.

Table 3 contrasts *Ranunculus septentrionalis* with its Pacific Coast and Rocky Mountain relatives:

TABLE 3

	R. septentrionalis	*R. orthorhynchus*	*R. Bloomeri*
Stem habit	Stoloniferous or often so	Never rooting	Never rooting
Radical leaf leaflet No.	1–3	3–7	3–5
Sepal habit	Spreading or rarely reflexed	Reflexed	Reflexed
Comparative length of sepals to petals	Two-thirds or three-quarters	One-third or one-half	One-half or two-thirds
Pubescence	Usually subglabrous but occasionally hispid	Hispid	Subglabrous

16A. RANUNCULUS SEPTENTRIONALIS var. **pterocarpus** L. Benson, var. nov.

Subglabrous; more markedly stoloniferous than the typical species; radical leaves simple or of 3 leaflets, these more shallowly lobed and toothed than in the typical species, crenate or serrate; sepals reflexed or spreading; achenes 5–15, the bodies obovate to suborbicular, 3.5–4.3 mm. long, 3.3–4 mm. broad, including the winged keels, which may be up to 1 mm. broad, the achene beaks about 2 mm. long; fruiting receptacle 4–5 mm. long.

Herba palustris perennis subglabra; stolonifera; dentibus foliorum

brevibus; acheniis 5–15, obovatis vel suborbicularibus, 3.5–4.5 mm. longis, 3.3–4 mm. latis, alatis; receptacula 4–5 mm. longo.

Marshy ground at low elevations; Wyandotte County, Kansas to Illinois, Indiana, Kentucky, and Tennessee; occurring as an intergrade to *R. palmatus* in southern Alabama and Florida; Dallas County, Texas. Southern deciduous and river bottom forests. May and June.

Type collection: Wet soil, Red River bottom near Boss, McCurtain County, Oklahoma, *H. W. Houghton 3732½*, May 23, 1916. TYPE in the New York Botanical Garden. An isotype is in the Gray herbarium.

Representative specimens: KANSAS: Wyandotte County, *Hitchcock 1105*, NY, GH. MISSOURI: St. Louis, *Riehl 25* in 1838, NY, *Eggert* in 1877, GH; Campbell, Dunklin County, *Palmer 39066*, GH. ILLINOIS: Mound City, HGr *16775*; Macon County, *Clokey 2384*, GH; Starved Rock, La Salle County, *Greenman, Lansing, & Dixon 123*, NY, GH. INDIANA: *Torr. & Gray, Fl. N. Amer.*, GH. KENTUCKY: Near State Lake, *Shacklette 273*, NY. TENNESSEE: Humboldt, Tennessei occidentalis, *Bain 383*, NY. OKLAHOMA: Boss, McCurtain County, Red River bottom, *Houghton 3732½*, NY, GH. TEXAS: Dallas County, *Reverchon 2739*, NY; Dallas, *Reverchon* in 1874, GH; San Felipe, *Lindheimer* in 1844, GH; Brazos River, *Drummond* in 1835, GH; Columbia, *Bush 147* GH; Blooming Grove, *Reverchon 3700*, GH. ALABAMA: Catoma Creek, Montgomery County, *Harper 85*, NY, GH. FLORIDA: Ocklocknee River, Leon County, West of Tallahassee, *Small & Wherry 11670*, GH, NY.

17. RANUNCULUS HISPIDUS Michx. Fl. Bor. Amer. **1**: 321. 1803. *Ranunculus marilandicus* Poir. in Lam. Encyc. Meth. **6**: 126. 1804 (?). *Ranunculus palmatus* Ell. Sketch. **2**: 61. 1816. *Ranunculus repens* L. var. *hispidus* Chapm. Fl. S. U. S. Ed. 1. 8. 1860. *Ranunculus septentrionalis* Poir. var. *marilandicus* Chapm. Fl. S. U. S. Ed. 3. 8. 1897 (?). *Ranunculus hirtipes* Greene, Ottawa Nat. **16**: 32. 1902.

Hirsute terrestrial perennials; roots usually fleshy, commonly 1.5–2.5 mm. in diameter; stems 1.5–3 mm. in diameter, fistulose, hirsute, the hairs sometimes appressed especially in specimens from Ontario, New York, and eastern Pennsylvania; radical leaf blades nearly always pinnately compound, cordate-ovate in outline, laciniate, 4–9 cm. long, 5–11 cm. broad, the terminal leaflet 3- and the lateral leaflets 2-parted, the parts sharply lobed or serrate-laciniate-toothed, blades proximally subcordate and distally acute, appressed-hirsute, the hairs rarely abundant; each achene obovate, 2.3–2.9 mm. long, 1.8–2.3 mm. dorso-ventrally, 0.7 mm. laterally, the achene beak slender, 1–2 mm. long, straight.

Moist places in meadows and rich woods at low elevations; Valley City, North Dakota, and Newark, Nebraska, to Ontario, Massachusetts, Missouri, Arkansas (modified form), Tennessee, and New Jersey; less common southward along the Atlantic Coast to Hendersonville, North Carolina and Atlanta, Georgia. Mostly Southern deciduous forest. April and May.

A form with radical leaves 8–10 cm. long and 12–16 mm. broad occurs in McDowell and Randolph Counties, West Virginia (Spruce Mountain

Branch of the Condon Lane Boom and Lumber Company Railroad, *Moore 2225*, GH; Railroad track, Allegheny Mountains, Randolph Co., *Greenman 366*, GH; Welch, *Webb* in 1922, GH). The leaf size and type are approached by plants from other parts of the west side of the Appalachian system. The leaf divisions are from narrowly elliptic to obovate. Segregation of varieties in *R. hispidus*, which is a polymorphic species, is difficult. The two listed below are well-distinguished in their extreme forms, but countless intermediate forms occur. From herbarium sheets distinction of *R. hispidus* from *R. septentrionalis* is not (apparently) always possible.

Type collections: (1) *R. hispidus*, "HAB. in umbrosis sylvis Carolinae inferioris." (2) *R. marilandicus*, "Cette plante croîte au Maryland . . . in herb. Bosc." This plant may be var. *falsus*. (3) *R. palmatus*, "Grows in St. John's Berkley." Near Savannah, Georgia. (4) *R. hirtipes*, "Obtained in woods near Sandwich, Ontario, 5 June, 1901, by Mr. John Macoun . . . bearing the Canad. Geol. Survey number 33,582." The TYPE is HGr. 2619.

17A. RANUNCULUS HISPIDUS var. FALSUS Fern. Rhodora **22**: 30. 1920. *Ranunculus cardiopetalus* Greene, Ottawa Nat. **16**: 32. 1902.

Stems 1–2 mm. in diameter, almost always appressed-pubescent, the hairs softer than in the typical species; the stems less markedly fistulose than in the typical species; radical leaf blades either simple and 3-parted or, if compound, the leaflets usually sessile and close to one another, less dissected than in the typical species, usually 1.5–4 mm. long, 2–5 mm. broad, but occasionally larger; fruit smaller than in the typical species and with a narrower keel (as pointed out to the writer by M. L. Fernald).

Dry or rocky woodland at low elevations; occasional from Toledo, Ohio to Pickett County, Tennessee; Ontario (Niagara Falls) to southwestern Vermont, Massachusetts, and Washington, D. C.; rare and modified in Virginia, North Carolina, and Alabama. Northeastern and Southern deciduous forests. April and May.

This variety is marked by small and most commonly simple leaves and by appressed pubescence and small fruit. Other forms are looked upon as intermediates, that is, if they show one of the characters and not the other.

Type collections: (1) *R. cardiopetalus*, "At the whirlpool Rapids, Niagara, Ont., 21 May, 1901, John Macoun (n. 33,581)." The TYPE is *HGr. 2546*. It has emarginate petals. (2) Var. *falsus*, "Dry wooded calcareous bank, Sheffield, May 30, 1919, Bean & Fernald. (TYPE in Gray Herb.)"

17B. RANUNCULUS HISPIDUS var. **eurylobus** L. Benson, var. nov.

Stems 1.2–2 mm. in diameter, very densely hirsute, the spreading hairs 2 mm. long; radical leaf blades mostly simple, the leaflets when present sessile and usually close to one another, the simple leaves lobed or divided, the leaflets or lobes or the whole leaf tending to be very broad or even orbicular, rounded at the bases, the leaf or leaflet 2–5 cm. long, 2–5 cm. broad.

Caulibus valde hirsutis; foliis vel foliolis latissimi vel orbicularibus, a basibus rotundis, 2–5 cm. longis, 2–5 cm. latis.

Hill country toward the interior from West Virginia and from Arlington, Brunswick County, and Fort Myer, Virginia, to Georgia and Alabama; Monterey, Tennessee; occurring in modified form in Kentucky and Tennessee and along the southern Atlantic coastal plain. March and April.

Type collection: "Oak woods, dry, Augusta, Georgia, March 26, 1899." TYPE in the New York Botanical Garden. Unfortunately the collector's name is not on the specimen, which is labelled in pencil. Although the variety has been collected rather frequently, no other specimen is suitable for a type.

Significant specimens: TENNESSEE: Monterey, *Cain & Sharp 4352,* NY. ALABAMA: Warrior River, Tuscaloosa, *Harper 3014,* NY. WEST VIRGINIA: Milton, Cabel County, *L. Williams 411,* NY. VIRGINIA: Arlington, *Blanchard* in 1891, NY; Meherrin River, 8 miles southeast of Cohran, Brunswick County, *Fernald & Long 7027,* GH; Fort Myer, *Mearns* in 1895, NY. NORTH CAROLINA: Tryon Mountain, Polk County, *Huger* in 1896, NY; Roanoke River at Weldon, *Small* in 1897, NY. SOUTH CAROLINA: Clemson College, Oconee County, *House 1712,* NY; Andersonville, *Earle* in 1886, NY. GEORGIA: Large Knob, Kenesaw Mountain, *Perry & Myers* in 1934, NY; Thompson Mills, Gwinnett County, *Allard 47* in 1908, NY.

18. RANUNCULUS FASCICULARIS Muhl. ex Bigel, Fl. Bost. Ed. 1. 137. 1814. *Ranunculus fascicularis* var. *Deforestii* Davis, Minn. Bot. Studies, **2**: 470. 1900. *Ranunculus illinoensis* Greene, Pittonia **5**: 195. 1903.

Stems 1–2 dm. long; radical leaf blades compound or the early ones 3-parted, ovate-oblong in outline, 2.5–5.5 cm. long, 2–4 cm. broad, distinctly longer than broad, leaflets 3 or commonly 5, sometimes the leaf partly bipinnate, the leaflets deeply 3–7-parted and again angularly-toothed, the ultimate parts blunt at the apices; petals 5 or rarely 9 (1 specimen), 7–15 mm. long; each achene obovate-orbicular with a short, flat stalk, the main body 1.5–2.5 mm. long, 1.5–2 mm. dorsoventrally, 0.6–1 mm. laterally, the margin keeled but not prominent.

Rather dry situations at low elevations; Taylor Falls, Minnesota, to southern Ontario, New England (except Maine and Rhode Island), New Jersey, and Pennsylvania (Lancaster) and southward to Oklahoma and Tennessee; "Southern Flora" (*Chapman,* NY; sepals reflexed). Northeastern and Southern deciduous forests. March to May, depending upon latitude; commonly called "early buttercup."

Truly tuberous roots are rare in specimens from east of the Mississippi Valley.

Type collections: (1) *R. fascicularis,* "The plants described in this book have been collected during the two last years in the vicinity of Boston, within a circuit of from 5 to 10 miles." 1812–13. (2) Var. *Deforestii,* "Collected by Henry P. DeForest (G. 42) near Rossville, Ill., April 12, 1885." The type specimen in the herbarium of Cornell University was kindly loaned to the writer by Dr. K. M. Wiegand. Some of the flowers have 9 petals and some have 5. (3) *R. illinoensis,* "Collected at Alto Pass, in south-

ern Illinois, 21 April, 1900, by Mr. Carl F. Baker, who reports that it occurs in large patches on moist open hillsides." Possibly a variety, but the type must be reexamined for characters other than the merely lobed early radical leaves, before a segregation can be made, if it is desirable. TYPE: *HGr. 2630.*

18A. RANUNCULUS FASCICULARIS var. APRICUS (Greene) Fern. Rhodora **38**: 178. 1936. *Ranunculus apricus* Greene, Pittonia **4**: 145. 1900.

Stems 1–3 dm. long; leaflets or leaf segments oblanceolate to narrowly elliptic, shallowly few-toothed apically or entire; petals 5, like those in the typical species; achenes like those of the typical species.

Mostly in the prairie country from Cherokee County, Kansas, to eastern Texas, Louisiana, and North Carollton, Mississippi; "S. W. Colorado" (*Flint,* NY). Prairie grassland; deciduous and river bottom forests. March and April.

The form occurring in wet ground in Louisiana and at Galveston, Texas is 2–3 dm. tall and is with usually small fruit and with less hair on the plant.

Type collection: "Near Sapulpa, Indian Territory Oklahoma, 29 April, 1895, B. F. Bush." The TYPE is *Bush 965, HGr. 2493.*

18B. RANUNCULUS FASCICULARIS var. **cuneiformis** (Small) L. Benson, comb. nov. *Ranunculus cuneiformis* Small, Bull. Torrey Club **27**: 276. 1900.

Stems 18–25 cm. long; leaves like the typical species, but a little larger and coarser; petals 7–9, 13–15 mm. long when fully expanded; achene 3 mm. long by 2.5 mm. dorsoventrally, the margin 0.5 mm. broad, distinctly marked.

Moist ground; collected at Bear Creek, Kerr County, Texas, and at Natchitoches, Louisiana (Palmer 6988). Southern deciduous forest. Early spring.

Type collection: "On prairies, near Kerrville, Texas. Spring. Heller, Pl. S. Tex. no. 1688."

DEPARTMENT OF BOTANY, UNIVERSITY OF ARIZONA
TUCSON, ARIZONA

NOTES ON POLYGONUM (AVICULARIA)

J. F. Brenckle

(WITH THREE FIGURES)

PHOTOPERIODISM AND TAXONOMY

Field observations in South Dakota show that the genus *Polygonum* is strongly affected by long days of bright sunlight. Profound changes take place in the plants during late June and in July, our longest and brightest days. The large spring stem-leaves fall from the plants and fruit production is slowed or stopped entirely. While each species reacts somewhat differently, the phenomenon takes place in all forms, both early and late. The small wiry species such as *P. achoreum* and *P. buxiforme* set fruit very early, often in the axils of the second and third stem-leaf, and fruit production continues until the latter part of June when the stem leaves and fruit fall away. Some of these plants do not survive this change, but others more favorably located continue growth with shortened internodes, smaller leaves, and a more compact inflorescence. *Polygonum* species with a late habit of growth, such as the group *P. ramosissimum* which seldom blooms in June with us, also lose the basal leaves but continue growth with narrower and smaller leaves through July; blooming is delayed to late August and September. Protection from intense or prolonged periods of sunlight by cloudiness or shade may prevent this retardation of bloom. Polygonums in general from high elevations show no such retardation. A series of specimens of *P. ramosissimum* collected on July 31 and August 1, 1939, along a road from Forsythe, Montana, to Mellette, South Dakota, show a marked difference in development. The western plants are more advanced, although collected from a higher elevation and latitude than those from the eastern half of South Dakota. Specimens from a creek-bed with perpendicular walls from 10 to 15 feet high, near Miles City, Montana, had well ripened achenes. These plants were the best developed of the series, no doubt owing to the protecting shade of the sidewalls. The winding arroyo with its walls afforded shade from east, west, or south, up to 30 to 40 per cent of the day's sunlight. Six specimens of the series are herewith deposited in the herbarium of the New York Botanical Garden.

Specimens of *Polygonum* collected in midsummer during the "rest period" are often sterile or otherwise in poor condition to classify. The marked difference between the spring, summer, and fall forms is confusing unless a series of specimens is at hand to represent these stages. Abundant and well developed material of *P. rubescens* Small sent me from Alberta, Canada, by Dr. Geo. H. Turner, illustrates this. The spring leaves have a

shape and size quite distinctive, are broadly elliptic or obovate, abruptly pointed at the apex, somewhat narrowed toward the base, subsessile or with a short petiole, 2–6 cm. long, 1–2.5 cm. wide. These merge into the narrow lanceolate leaves of summer and are lacking in fruiting specimens. Specimens in triplicate representing spring, summer, and fruiting stages of this plant will be deposited in the Gray Herbarium, the National Herbarium and the herbarium of the New York Botanical Garden.

THREE ALIEN POLYGONUMS IN THE WEST

POLYGONUM ARGYROCOLEON Steudel. "Silver-sheath knotweed."[1] "Erect, annual, with slender branches; leaves linear-lanceolate, often deciduous; flowerspikes slender, naked; flowers pedicelled, shortly exserted from the ocreolae; perianth about 2 mm. long; achenes equal to the perianth, brown, smooth, and shining."[2] The plant has a dark grey-green color, strikingly different from that of American species. The silvery ocreae are membranous, showy, and white. Introduced into southern California and Arizona with alfalfa seed from Persia and widespread in alfalfa and grain fields.[1]

Specimens examined: California, Santa Catalina Island, *Fosberg 4461* (as *P. ramosissimum*): Imperial County, alfalfa fields; *Bellue*, July 6, 1932.

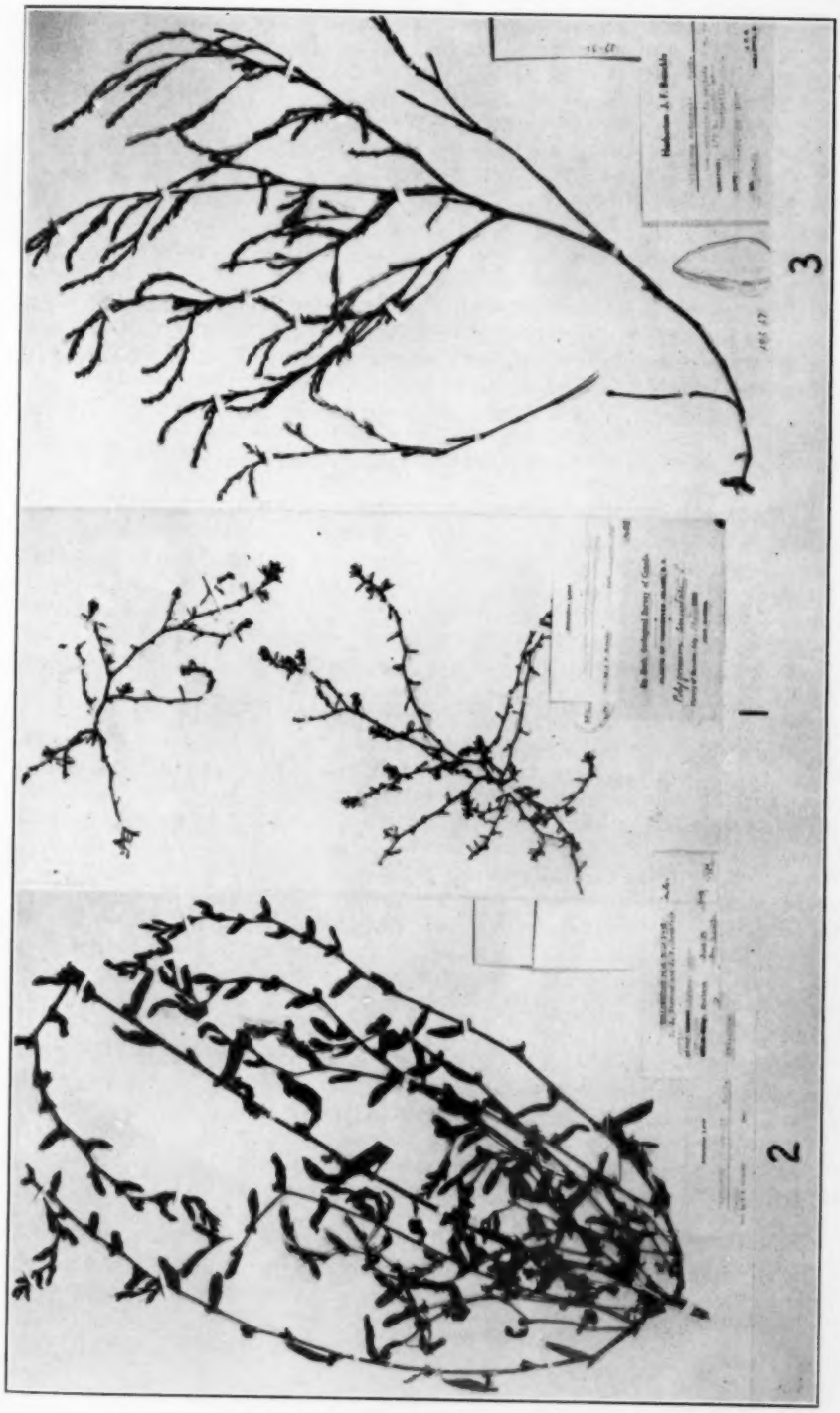
POLYGONUM HUMIFUSUM Pall. ex Ledeb. (not *P. humifusum* Jord.). "A smooth, branching, prostrate herb; leaves linear to oblong, obtuse; inflorescence at tips of the branches, fascicles 4–10 flowered; achenes smooth, exceeding the perianth."[2] A skeleton-like plant with spathulate-linear leaves mostly divergent or reflexed; flowering from the base in the axils of all leaves; achenes lenticular or three-angled, ovate, pointed, black, smooth, and shining, about 1 mm. long (fig. 1). Three sheets of this species have been examined, all collected near Nanaimo, Vancouver Island, B.C., by *Dr. John Macoun*, and distributed under *No. 1483* (as *P. aviculare*). The sheet in the Canadian National Herbarium, Ottawa, was collected July 11, 1887, "on ballast." This plant, about 5 to 6 dm. long, has all its achenes 3-angled. A sheet in the herbarium of the University of Minnesota, Minneapolis, has three plants of about half the length of the above, collected on July 12, 1893, six years later. These have all achenes lenticular, pointed, black, smooth, and shining, about 1 mm. long. The third sheet, in the herbarium of the New York Botanical Garden, dated July 12, 1893, Nanaimo, B. C., has two plants similar in size to the Minnesota specimens, one of which has its achenes 3-angled and the other lenticular. These remarkable plants appear to belong to the section *Avicularia*. No known American species of this section has lenticular achenes. Among the *Persicariae* some species may have occasional 3-angled achenes, but the plants here represented have them either lenticular or 3-angled, with no mixture on a single plant.

[1] Bellue, Margaret K. 1935, Silver-sheathed Knotweed as a pest in S.W. Alfalfa. Mo. Bull. Dept. Agr. Calif. June 1935: 238–241.

[2] Steward, Albert N. The *Polygoneae* of eastern Asia. Contr. Gray Herb. 87: 19. 1930.

Explanation of figures 1–3

FIG. 1. *Polygonum humifusum*. FIG. 2. *Polygonum Stevensii*. FIG. 3. *Polygonum autumnale*.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POLYGONUM POLYCNEMOIDES Jaub. et Spach. "A prostrate branching herb with appressed linear-subulate leaves: ocreae hyaline, white, conspicuous, sometimes lacerate: flowers axillary, near the tips of the branches, sessile, about 2 mm. long: achenes triangular, black, included in the perianth; faces minutely punctate. South-central Asia."[3]

The specimens kindly sent me by Prof. Ray J. Davis, from Idaho, collected on an abandoned field, no doubt belong to this Asiatic species and were introduced with garden or field seed. The striking character which differentiates them from American species is the perianth. Grey-green, scurfy, constricted above, closely investing the mature achene; shallowly 5-parted to about one-fourth its length; the limb is spreading, white, .4–.5 mm. long. Sometimes a short white-hyaline, denticulate, ocreolus persists about the base of the perianth. The dry stems are fragile and the leaves veinless and glandular-punctate. The rather short limb probably places these plants with *P. Oliveri* Jaub. et Spach.[3]

No. 2833, Ray J. Davis, Idaho, is deposited in the New York Botanical Garden.

TWO NEW SPECIES

Polygonum Stevensii Brenckle, sp. nov. Planta annua; caulis acute angulatus, erectus, 4–7 dm. altus, base ramosus; folia crassa, oblonga, brevipetiolata, inconspicue nervata, 2–5 cm. longa, 4–10 mm. lata, tam longa vel longiora quam internodi. Ocreae inconspicuae, macrae; flores copiosi; perianthi axillares, 2–8 ad quodquam folium, pedicellati, 4–5 mm. alti. cum sex segmentis; segmenta tres exteriora carinata, nervata, cucullata, viridia, albomarginata; achaenia 3-angulata, fusco-atrata, glabra, muriculata, 3.5 mm. longa.

Annual; plant yellowish green throughout, stem smooth, striate, sharply angled, erect, 4–7 dm. high, profusely branched at the base with several elongated, decumbent or prostrate branches; stem-leaves thick, smooth, oblong or oblanceolate, rounded or bluntly pointed at the apex, tapering to a short petiole at the base, veins inconspicuous or none except the midrib at the back, 2–5 cm. long, 4–10 mm. wide, often widest above the middle, as long or longer than the internodes, articulate to the foliaceous part of the ocreae; ocreae thin, scarious, with few or no veins, inconspicuous; flowers abundant, 2–8 in the axil of each leaf; perianth segments 6, the outer three sharply carinate, prominently veined, cucullate, green with white edges; style short, 3-parted to the base; stamens 5, yellow; achenes ovate, prominently 3-angled, angles rounded, becoming black, muriculate, 3.5 mm. long, 2 mm. wide.

Specimens examined: The type was collected by Brenckle and Stevens on June 29, 1938, on low ground near a spring adjoining the highway, south of Forsythe. Montana (fig. 2). Specimens (*No. 38–034, Brenckle & Stevens*) are deposited in the Rocky Mountain Herbarium, Laramie, Wyoming; University of Wisconsin Herbarium, Madison; Herbarium of the N. Y. Botanical Garden (TYPE); U. S. National Herbarium, Washington.

This plant is related to *P. ramosissimum* Michx. but differs in the stem, in the manner of branching, in the character and shape of the leaves, and in

[3] Steward, Albert N. *l.c.*: 25, 26.

the early and abundant inflorescence. It differs from *P. latum* Small in the manner of branching, shape and character of leaves and perianth, and by the early bloom.

Polygonum autumnale Brenckle, sp. nov. Annua; caulis erectus, gracilis, 2–5 dm. altus; folia erecta, lanceolata vel linearia, ad apice et base acuminata, 2–4 cm. longa, 2–4 mm. lata, cito decidua; florit aestate sera; perianthi sessiles, ocreis celati; achaenia 3-angulata, anguste ovata, obtuse apiculata, latera plana, fusca, subtiliter punctata, hebetia, 1.6 mm. longa, 0.8 mm. lata.

Annual; stem mostly erect, slender, wiry, terete, finely striate branches erect or spreading below, 2–5 dm. high; spring leaves erect, lanceolate or linear, veined beneath, parallel lined above when dry, pointed at each end, subsessile, pale green, 2–4 cm. long, 2–4 mm. wide, early deciduous, reduced above to linear bracts; ocreae veined, the lower half herbaceous, the upper membranous part evanescent leaving the exposed veins as bristles; inflorescence develops late in summer on terminal branches and twigs, interrupted or contiguous and spike-like; perianth sessile or subsessile, 1–3, mostly hidden in the ocreae, 5-parted, covering the achene; segments green or reddish tinted with white or white and pink margins; achenes obtusely 3-angled, narrowly ovate, obtusely pointed at the top, faces mostly flat or slightly convex, brown, muriculate or minutely striate, dull 1.2–2 mm. long, about .8 mm. wide. Rarely a few blossoms develop in spring.

Specimens examined: The TYPE, *No. 37–32*, collected near Northville, Spink County, South Dakota, September 23, 1937, is deposited in the herbarium of the New York Botanical Garden (fig. 3). The following specimens are in the U. S. National Herbarium.

SOUTH DAKOTA: Rapid City, *D. Griffiths No. 732*, Aug. 29, 1897 (3 sheets). TEXAS: Bell County, *Simon E. Wolff No. 175*, Oct. 17, 1928; Amarillo, *Carlton & Ball No. 1136*, Sept. 15, 1906. MARYLAND: Calvert County, Cheseapeake Beach, *N. D. House No. 1454*, Aug. 20, 1905.

At hand for distribution to those interested are additional specimens of cotype material from South Dakota and North Dakota; Burleigh County, McKensie, *O. A. Stevens No. 485*, Aug. 15, 1940.

This species, practically sterile all summer, has been overlooked or confused with other species. The early loss of the stem leaves and variation in size of all parts according to location, crowding and moisture, make it difficult to present a complete picture of the plant. It has been variously named *P. aviculare*, *P. prolificum* and *P. leptocarpum*, but from these it differs in the perianths hidden in the ocreae, narrower ripe achene often square-cut on the edges, stub-pointed and mostly less than 2 mm. long, usually from 1.2 mm. to 1.6 mm. long. Late in the season we may find a few exserted achenes which are longer.

The writer wishes to gratefully acknowledge the assistance and advice freely given by Prof. O. A. Stevens of Fargo in preparation of this paper; also to Mr. L. L. Shinners of the University of Wisconsin, Madison, for the Latin diagnoses.

AN UNDESCRIBED LENOPHYLLUM[1] FROM MEXICO

STEPHEN S. WHITE

(WITH ONE FIGURE)

Lenophyllum reflexum White, sp. nov. Folia opposita, late ovata vel elliptica, ad 4.5 cm. longa et 3 cm. lata, acuta, supra plana vel convexa, pleraque reflexa. In vivo folia matura supra purpureo-viridia, infra purpurea, in sicco viridia; juvenile in vivo lucide viridia. Inflorescentia paniculata ramulis tenuibus, siccata rubeo-purpurea. Flores remoti, fere sessiles, bracteis deciduis 3 mm. longis subtenti. Perianthii flavo-virides, siccati saepe rubeo-purpurei. Sepala 4 mm. longa, oblonga, acuta. Petala 6 mm. longa oblanceolata acuta. Stamina 10. Filamenta petala opposita duplices quam longitudine antherae, ad inferiorem tertiam partem petalorum inserta. Carpella 5–6 mm. longa, stylis subulatis, squamis carnosis et truncatis.

Succulent perennial herb. Leaves opposite, broadly ovate to elliptic, up to 4.5 cm. long and 3 cm. wide, acute, flat or convex above, mostly reflexed. Mature leaves on living plant suffused with purple, especially below; young leaves bright green. Inflorescence paniculate, many-flowered, with slender branches, turning reddish-purple when dry. Flowers remote, nearly or quite sessile, subtended by deciduous bracts 3 mm. long. Perianth yellowish-green; sepals 4 mm. long, oblong, acute; petals 6 mm. long, oblanceolate, acute. Stamens 10, the filaments of those opposite the petals becoming twice as long as the anthers, inserted at one-third the height of the petals. Carpels 5–6 mm. long with subulate styles; scales fleshy and truncate.

TYPE in the University of Michigan Herbarium, prepared from a plant grown in the Botanical Gardens of the University of Michigan; collected by *H. H. Bartlett* in 1930 on the Cerro del Diablo, San Carlos Mts., Tamaulipas, Mexico.

This species is clearly distinguished from all other members of the genus by its broad reflexed leaves which are not at all channeled above.

The author wishes to acknowledge his indebtedness to Drs. E. B. Mains and C. L. Lundell of the University of Michigan Herbarium for many valuable suggestions in the preparation of the manuscript.

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[1] Family Crassulaceae.

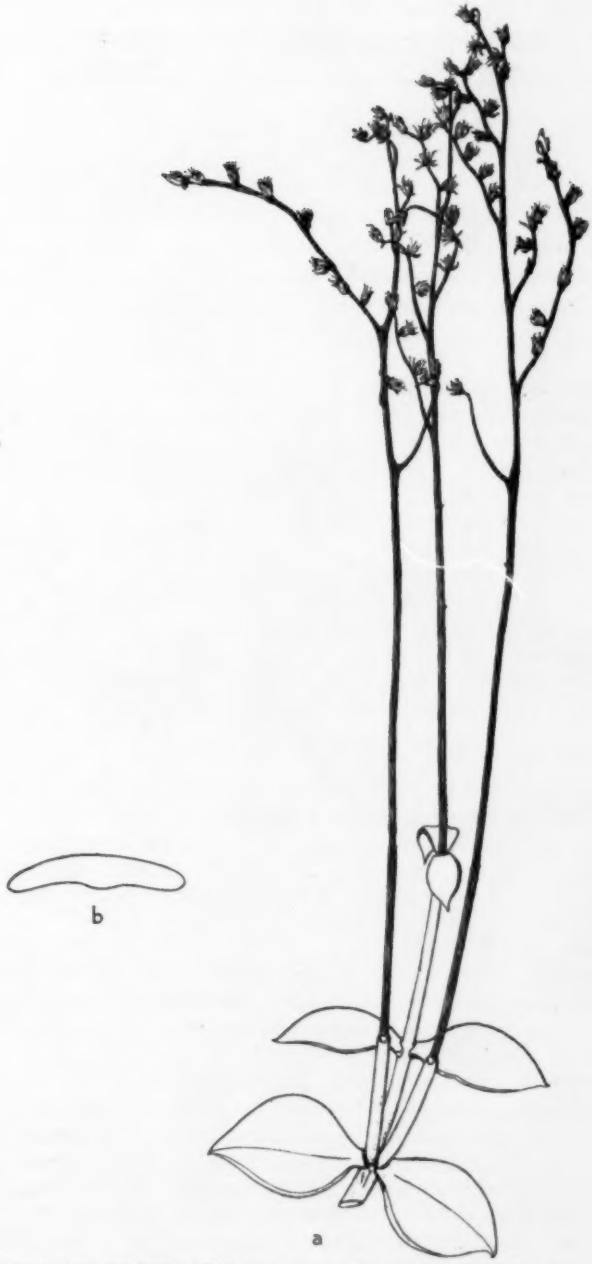

FIG. 1. *Lenophyllum reflexum* White, sp. nov.; *a*, habit, one-half natural size; *b*, cross section of leaf, natural size.

NEW SPECIES AND VARIETIES OF VERBENACEAE FROM CENTRAL AND SOUTH AMERICA

HAROLD N. MOLDENKE

Continued work on the *Verbenaceae* has brought to light a number of as yet undescribed species and varieties from tropical America. It has been thought advisable to place the descriptions of these plants formally on record at this time. Detailed discussions of their relationships and distribution, and complete citation of specimens, will be published at a later date in generic monographs now in preparation.

Bouchea boyacana Moldenke, sp. nov. Herba perennis sublignosa; ramis ramulisque dense puberulis; petiolis crassiusculis complanatis alatis dense puberulis; laminis firme chartaceis ovatis, ad apicem et basim acutis, crasse dentatis utrinque dense adpresso-puberulis; pedunculis rhachideque pedicellisque dense puberulis.

Rather woody perennial; branches and branchlets rather slender, obtusely or subacutely tetragonal, often rather irregular, densely puberulent throughout; nodes not annulate; principal internodes usually abbreviated, 3–15 mm. long; leaves decussate-opposite; petioles rather stout, 1–1.5 cm. long (or less), flattened above and alate from apex to base with a narrow green marginal wing about 1 mm. wide or less, densely puberulent throughout; blades firmly chartaceous, ovate, almost uniformly light-green on both surfaces or somewhat darker above and grayish-green beneath, 1–5 cm. long, 0.5–3.7 cm. wide, acute at apex, coarsely dentate from the widest part to the apex with broadly ovate acute or apiculate antrorse teeth to 7.5 mm. long and 4 mm. wide, acute at base, densely appressed-puberulent on both surfaces, the puberulence often grayish beneath; midrib slender, prominulous on both surfaces; secondaries slender, 2–5 per side, ascending, only slightly arcuate, often extending from near the base to the apex, subprominulous on both surfaces; veinlet reticulation obscure or indiscernible; inflorescence terminal, spicate during anthesis, racemiform in fruit, 4–37 cm. long, many-flowered, erect, the flowers usually imbricate or the lowermost separate; peduncles slender, abbreviated, 1 cm. long or less, along with the rachis densely puberulent; pedicels subobsolete during anthesis or to 1 mm. long, in fruit to 4 mm. long and densely puberulent; foliaceous bracts absent; bractlets and prophylla lanceolate, 1–3 mm. long, attenuate, densely puberulent; calyx tubular, 6–9 mm. long, 1.5–2.5 mm. wide throughout, densely puberulent outside, 5-costate, subhyaline and translucent between the costae, its rim 5-apiculate, the apiculations unequal—four 1–1.5 mm. long and the fifth 0.5–0.9 mm. long, cucullate from within; corolla hypocrateriform, irregular, its tube broadly cylindric, curvate, about 1.5 cm. long, about 1 mm. wide at base, ampliate to 3 mm. somewhat below the apex, glabrous outside, pilose among the stamens within, its limb spreading, 5-parted, 2-lipped, the lobes broadly elliptic, rounded at apex, the largest 6–6.5 mm. long and wide; stamens 4, didynamous, included, inserted about 3 and 4 mm. below the mouth of the corolla-tube, the lower pair shorter; filaments filiform, about

1.4 and 2.4 mm. long, glabrous or the larger pair obscurely pilose; anthers broadly ovate-elliptic, about 1 mm. long and wide, the connective ovate-triangular; pistil included; style capillary, about 9 mm. long, glabrous; stigma 2-lobed, the anterior lobe club-shaped and erecto-recurved, the posterior lobe minute and tooth-like; ovary elongate-oblong, about 2.5 mm. long and 1 mm. wide, glabrous.

COLOMBIA—BOYACÁ: Cordillera Oriental, Soatá, arid slopes, alt. 2130 m., *J. Cuatrecasas 1017*, September 6, 1938, TYPE; in the Britton Herbarium at the New York Botanical Garden.

Duranta armata Moldenke, sp. nov. Frutex; ramis ramulisque rigidis sarmentosis spinosis adpresso-puberulis glabrescentibus; foliis fasciculatis numerosis subsessilibus vel brevissime petiolatis; laminis chartaceis oblanceolatis vel ellipticis acutis vel obtusis vel rotundatis integris vel 2–4-dentatis, ad basim acutis vel attenuatis, utrinque sparse minuteque puberulis glabrescentibus, supra nitidis, subtus punctatis; inflorescentiis abbreviatis terminalibus 1- vel pauci-floris.

Spiny shrub; branches and branchlets medium-slender, stiff, twiggy, obtusely tetragonal or subterete (sharply tetragonal when young), rather densely appressed-puberulent when young, glabrescent in age, viciously spinose; spines numerous, slender to very stout, alternate-scattered, rarely opposite or ternate, 0.9–3.3 cm. long, very stiff and sharp-pointed, unbranched, often bearing an opposite pair of buds at about the middle, glabrous and shiny; nodes not annulate; principal internodes abbreviated, 1–2 cm. long or less; leaves borne in fascicles on very much abbreviated spur-like twigs subtending the spines, numerous, subsessile or very short-petiolate; petioles slender, 1–3 mm. long or obsolete, appressed-puberulent, submargined; blades chartaceous, oblanceolate or elliptic, 0.4–3 cm. long, 4–14 mm. wide, varying from acute (on large leaves) to obtuse or rounded (on small leaves) at apex and often subemarginate, entire or with 1 or 2 appressed irregular teeth on each margin near the apex, acute or attenuate at base, sparsely and minutely puberulent on both surfaces when young, glabrous in age and shiny above and punctate beneath; midrib slender, flat or subimpressed above, prominulous beneath; secondaries slender, 2–5 per side, flat or subimpressed above, usually flat beneath; veinlet reticulation obscure or indiscernible; inflorescence reduced, terminating the branchlets and twigs, racemiform, 1–few-flowered, 1–5.5 cm. long; peduncles slender, sharply tetragonal, abbreviated, 2–18 mm. long, along with the rachis rather densely appressed puberulent; pedicels slender, about 1 mm. long, elongate to 4 mm. in fruit, puberulent; bractlets varying from linear to spatulate, stipitate, 1–4 mm. long, puberulent; calyx campanulate, 3–3.5 mm. long, 2.5–3 mm. wide at apex, 5-costate, appressed-puberulent outside, its rim truncate, shortly 5-apiculate; corolla blue, hypocrateriform, its tube broadly cylindric, about 7 mm. long, glabrous outside except at the very apex, straight or slightly curved, its limb 5-parted, 7–10 mm. in diameter, densely pulverulent-puberulent on both surfaces; fruiting-calyx broadly campanulate, papery, obvolute and subincluding the fruit, eventually splitting, minutely puberulent or glabrate and shiny, its rim usually persistently whitish puberulent-pilose; fruit tetragonal, about 4 mm long and wide, very distinctly 4-lobed and -sulcate in drying, glabrous and shiny.

PERU—CUZCO: Upa Blanca, near Cuzco, alt. 3400–3600 m., *Fortunato L. Herrera 85*, TYPE; in the herbarium of the Botanisches Museum at Berlin, received there in February, 1923.

Duranta Dombeyana Moldenke, Prelim. Alph. List Invalid Names 25, hyponym (1940), sp. nov. Frutex; ramis glabratis saepe sparse spinosis; ramulis dense fulvo-tomentosis; foliis oppositis; petiolis dense tomentosis glabrescentibus; laminis chartaceis ellipticis, ad apicem obtusis vel acutis vel subacuminatis, ad basim rotundatis vel acutis vel acuminatis, integris vel irregulariter serratis, supra sparse breviterque pubescentibus vel puberulis glabrescentibus, subtus dense adpresso-tomentosis; inflorescentiis axillaribus terminalibusque; floribus secundis; pedunculis rhachideque dense flavescento-tomentosis.

Strict shrub, 2–4 m. tall; branches rather slender, obtusely tetragonal, glabrate, sometimes sparsely spinose; spines axillary, opposite, stout at base, sharply pointed at apex, 5–7 mm. long; branchlets slender, obtusely tetragonal, densely fulvous-tomentose with short appressed tomentum; nodes not annulate; principal internodes 0.5–4 cm. long, those on the twigs extremely abbreviated; leaves decussate-opposite, often with an additional pair or a fascicle borne on an extremely abbreviated twig in the axil; leaf-scars corky, prominent, semicircular; petioles slender, 4–8 mm. long, margined above, densely tomentose or glabrescent; blades chartaceous, rather uniformly dark-green above or slightly lighter beneath, sometimes brunnescent above in drying, elliptic, 1.7–9.3 cm. long, 1.1–4 cm. wide, varying from obtuse to acute or even subacuminate (occasionally emarginate) at apex, entire or rarely rather irregularly serrate just below the apex or from the widest part to the apex with sharp-pointed antrorse teeth, varying from rounded to acute or acuminate at base and usually more or less prolonged into the petiole, lightly short-pubescent or puberulent above, glabrescent in age, densely appressed-tomentose with fulvous or yellowish hairs beneath; midrib slender, flat or subimpressed above, prominent beneath; secondaries slender, 4–6 per side, ascending, not much arcuate, flat or subimpressed above, prominulous beneath, joined in many loops near the margins; veinlet reticulation sparse, the larger portions subimpressed above and subprominulous beneath, the rest obscure or indiscernible; inflorescence axillary and terminal, the axillary ones 3–7 cm. long, the terminal one to 20 cm. long, spicate or subracemiform, many-flowered, the flowers secund; peduncles abbreviated, 4–13 mm. long, along with the rachis densely flavescent-tomentose; pedicels obsolete or to 2.5 mm. long and densely flavescent-tomentose; bractlets and prophylla linear, 1–6 mm. long, densely tomentose, caducous; calyx campanulate, 3–4 mm. long, 2–3.5 mm. wide, 5-costate, densely flavescent-tomentose outside, its rim truncate, very obscurely and minutely 5-apiculate; corolla hypocrateriform, its tube broadly cylindric, curved, 1.3–1.4 cm. long, glabrous outside below the bend, puberulent above, its limb 8–11 mm. in diameter, densely puberulent on both surfaces; fruiting-calyx obvolute and rostrate, completely enclosing the fruit, firm, nigrescent, short-pubescent with canescent hairs (especially on the apical beak), becoming glabrous and shiny in age, eventually splitting; fruit subglobose, to 1 cm. long and wide, glabrous, shiny, nigrescent in drying, umbonate-rostrate at apex.

ECUADOR: between Loja and Portovelo, *Joseph Nelson Rose, Abelardo*

Pachano, & George Munson Rose 23,347, October 3–6, 1918, TYPE; in the Britton Herbarium at the New York Botanical Garden.

Duranta guatemalensis Moldenke, sp. nov. Frutex; ramis ramulisque spinosis dense adpresso-tomentosis glabrescentibus; foliis oppositis vel subopositis plerumque fasciculatis; petiolis dense tomentoso-pubescentibus glabrescentibus; laminis tenuiter chartaceis vel submembranaceis oblanceolato-ellipticis vel ellipticis, ad apicem obtusis vel rotundatis, integris vel sparse serratis, supra parce pilosulis glabrescentibus, subtus puberulento-pilosulis; inflorescentiis axillaribus terminalibusque; pedunculis rhachideque dense adpresso-tomentosis.

Spiny shrub; branches and branchlets slender, obtusely tetragonal or subterete, densely appressed-tomentose with white or sordid tomentum when young, glabrescent in age; spines in opposite or subopposite pairs, stiff, slender, 2.5–14 mm. long, glabrous, sharp-pointed; nodes not annulate; principal internodes 1–3.7 cm. long; buds densely tomentose; leaves decussate-opposite or subopposite, often with additional fascicles on extremely abbreviated twigs in their axils; petioles very slender, 1–5 mm. long, densely tomentose-pubescent with appressed sordid hairs, glabrescent in age; blades thin-chartaceous or submembranous, oblanceolate-elliptic or elliptic, lighter beneath, 1.2–4 cm. long, 8–16 mm. wide, obtuse or rounded at apex, entire or sparsely serrate at or near the apex with a few, appressed, mostly rounded teeth, sparsely pilosulous above, glabrescent in age, rather sparsely puberulent-pilosulous beneath (somewhat more densely so on the larger venation); midrib very slender, flat or subimpressed above, flat or prominulous beneath; secondaries very slender, 3–5 per side, ascending, not much arcuate, flat or subimpressed above, flat or prominulous beneath, obscurely joined at the margins beneath; veinlet reticulation sparse, mostly indiscernible above and obscure beneath; inflorescence axillary and terminal, racemiform, 3–5 cm. long, many-flowered; peduncles very slender, 3–6 mm. long, along with the slender rachis densely appressed-tomentose like the branchlets; bracts and prophylla linear, 1–2 mm. long, densely tomentose, deciduous; calyx tubular, 5–7.5 mm. long, 2–3.5 mm. wide, densely or irregularly short-pubescent-pilose outside, less so in age, 5-costate, thin-textured between the ribs, its rim truncate and very minutely 5-apiculate; corolla hypocrateriform, its tube broadly cylindric, slightly curved, 7–10 mm. long, subglabrate below outside, densely puberulent toward the apex, its limb 5-parted, 9–11 mm. in diameter, very densely appressed-puberulent with canescent hairs on both surfaces.

GUATEMALA—QUICHÉ: Chiul, alt. 2600 m., *Enrique Teófilo Heyde & Ernesto Lux 2947*, in June 1892, TYPE; in the Gray Herbarium of Harvard University.

Duranta peruviana Moldenke, sp. nov. Frutex; ramis ramulisque dense breviterque pubescentibus glabrescentibus, ad nodos decussato-complanatis, saepe spinosis; foliis oppositis; petiolis dense breviterque incano-pubescentibus; laminis tenuiter chartaceis ellipticis, ad apicem acutis vel breviter acuminatis, integris vel serratis, supra dense puberulis, subtus dense velutino-pubescentibus; inflorescentiis axillaribus terminalibusque saepe paniculatis; pedunculis rhachideque dense breviterque incano-pubescentibus.

Shrub; branches and branchlets slender, obtusely or subacutely tetrag-

onal, decussately flattened at the nodes, densely short-pubescent when young, glabrescent in age, grayish, sometimes bearing a few pairs of short blunt spines above the nodes; nodes not annulate, mostly flattened and somewhat ampliate; principal internodes 2–4 cm. long; leaves decussate-opposite; petioles slender, 4–10 mm. long, densely short-pubescent with incanous hair; blades thin-chartaceous, uniformly dark-green on both surfaces, elliptic, 1.3–7 cm. long, 0.8–4.5 cm. wide, acute or short-acuminate at apex, entire or serrate with blunt appressed teeth from below the middle to the apex, densely puberulent above, densely velutinous-pubescent with sordid-grayish hair beneath; midrib slender, flat or subimpressed above, prominent beneath; secondaries very slender, 6–9 per side, arcuate-ascending, flat or subimpressed above, prominulous beneath; veinlet reticulation obscure or indiscernible above, often obscure beneath; inflorescence axillary and terminal, racemiform or paniculate, 7–14 cm. long, often bearing 1 or more pairs of opposite racemiform branches; racemes many-flowered, spreading; peduncles slender, 0.5–2.5 cm. long, usually abbreviated, along with the slender rachis densely short-pubescent with incanous hairs; pedicels very slender, obsolete or to 3 mm. long during anthesis and fruit, densely incanous-pubescent; calyx tubular-campanulate, 3.5–4 mm. long, about 2 mm. wide, 5-costate, densely strigose-pubescent with incanous hair, its rim truncate, minutely 5-apiculate; corolla hypocrateriform, its tube slightly curved, 6–8 mm. long, densely puberulent outside above the calyx, its limb 5-parted, to 1 cm. in diameter, densely incanous-puberulent on both surfaces.

PERU—CUZCO: between Santa Rosa and Quillabamba, Convención, *J. Soukup 825*, in January, 1938, TYPE; in the herbarium of the Field Museum of Natural History at Chicago.

DURANTA PERUVIANA var. **longipedicellata** Moldenke, var. nov. Haec varietas a forma typica speciei recedit pedicellis post anthesin usque ad 8 mm. longis, floribus nutantibus, ramulis foliisque sparsiore pubescentibus.

This variety differs from the typical form of the species in its pedicels being up to 8 mm. long after anthesis, the flowers nutant, and the branchlets and lower leaf-surfaces less densely velutinous-pubescent.

PERU—CUZCO: valley of the Urubamba, Machupicchu, alt. 2200 m., *Fortunato L. Herrera 3209*, in October, 1931, TYPE; in the herbarium of the Field Museum of Natural History at Chicago.

Duranta Skottsbergiana Moldenke, Suppl. List Vern. Names 10, hyponym (1940), sp. nov. Frutex vel arbor parva; ramis plerumque spinosissimis glabratis; ramulis dense breviterque pubescentibus glabrescentibus saepe spinosis; foliis oppositis vel ternatis vel irregulariter dispositis, saepe fasciculatis; petiolis marginatis breviter pubescentibus glabrescentibus; foliis subcoriaceis nitidis ellipticis vel lanceolatis vel oblanceolatis, ad apicem obtusis vel acutis vel subemarginatis, ad basim acutis vel acuminatis, integris vel sparse arguteque serratis, supra sparse pilosis glabrescentibus, subtus dense adpresso-pubescentibus dein glabrescentibus et impresso-punctatis; inflorescentiis axillaribus terminalibusque saepe paniculatis.

Shrub or small tree, to 6.5 m. tall; branches medium-stout, brown, subterete, glabrate, prominently lenticellate-verruculose, usually very spinose; branchlets medium, stiff, densely short-pubescent when young, glabrescent in age, often spinose, subterete or angular; spines very stiff, rather slender, 0.6–3.4 cm. long, very sharp-pointed, gray, glabrous, opposite, ternate, or

scattered; nodes not annulate, often triangular-flattened on younger parts; principal internodes 1–5 cm. long; leaves opposite, ternate, or scattered (on older branches), often borne in fascicles on greatly abbreviated twigs from below the spines on older wood; petioles slender, 2–11 mm. long, densely or sparsely short-pubescent, glabrescent in age, margined from apex to base, flat above; blades subcoriaceous, dark-green above, lighter beneath, shiny on both surfaces, mostly elliptic, varying to lanceolate or oblanceolate, 1.5–4.5 cm. long, 0.6–2.7 cm. wide, obtuse or acute at apex, varying to subemarginate, entire or sparsely sharp-serrate with appressed teeth toward the apex, acute or acuminate at base, often attenuate into the petiole, sparsely scattered-pilose above when young, soon glabrescent, densely appressed-pubescent beneath when young, eventually glabrous and impressed-punctate; midrib slender, flat above, prominent beneath; secondaries slender, 4–6 per side, arcuate-ascending, flat above, sharply prominent beneath, not anastomosing; veinlet reticulation sparse, obscure on both surfaces or indiscernible in mature leaves; inflorescence racemiform or paniculate, axillary and terminal, 2–19 cm. long, often bearing one or more pairs of racemiform branches near the base, arcuately ascending or recurved, many-flowered; flowers subsecund; bracts large and foliaceous, resembling the leaves in all respects but smaller, a pair subtending each pair of inflorescence-branches; bractlets elliptic or spatulate, stipitate, to 7 mm. long and 3 mm. wide, densely short-pubescent, borne near the base of the racemes, caducous; prophylla linear, minute, about 1 mm. long, densely pubescent; peduncles slender, 1–2 cm. long or more abbreviated, along with the slender rachis densely short-pubescent, glabrescent in age; calyx campanulate, 4–5 mm. long, 2.1–4 mm. wide, 5-costate, thin-textured between the ribs, densely puberulent or short-pubescent with appressed cinereous hairs, its rim truncate, minutely 5-apiculate; corolla hypocrateriform, pale-lilac, pale-blue, or lavender, its tube broadly cylindric, 1–1.4 cm. long, curved, the uppermost 3–4 mm. densely puberulent outside, the remainder glabrous outside, its limb 5-parted, 7–10 mm. in diameter, densely cinereous-puberulent on both surfaces; fruiting-calyx leathery, glabrate; fruit subglobose, yellow, about 1 cm. long and wide.

PERU: Mito, in sunny stream canyons, alt. about 3000 m., *J. Francis Macbride & Featherstone 1484*, July 8–22, 1922, TYPE; in the herbarium of the Field Museum of Natural History at Chicago.

This species is named in honor and appreciation of my respected colleague, Dr. Carl Skottsberg, Director of the Botanical Garden at Göteborg, Sweden, to whose boundless energy and inexhaustible courage this garden will always be a lasting memorial, and who has found time, amid all his administrative work, to do such noteworthy botanical exploration in Chile, the Galapagos Islands, Hawaii, and elsewhere.

Duranta Woronowii Moldenke, sp. nov. Frutex vel arbor; ramulis glabris inermibus; foliis oppositis vel suboppositis; petiolis debilibus minute obscureque puberulis vel subglabratis; laminis tenuiter chartaceis vel submembranaceis brunnescentibus anguste ellipticis, ad apicem acutis, ad basim cuneato-attenuatis vel subacuminatis, integris, utrinque minutissime obscureque subpilosulis glabrescentibus; inflorescentiis axillaribus terminalibusque racemiformibus; floribus non secundis plusminus erectis; pedunculis glabris.

Shrub or tree; branchlets slender, dark, obtusely tetragonal, lenticellate,

glabrous, unarmed; nodes not annulate, not ampliate; principal internodes 2.5–3.5 cm. long; leaves decussate-opposite or subopposite; petioles slender, weak, 5–10 mm. long, minutely and obscurely puberulent or subglabrate, canaliculate above; blades thin-chartaceous or submembranous, dark-green above, lighter beneath, brunnescent in drying, narrow-elliptic, 4.5–8 cm. long, 1.2–2.5 cm. wide, acute at apex, entire, cuneate-attenuate or subacuminate at base, very minutely and obscurely subpilosulous on both surfaces or glabrescent; midrib slender, flat above, prominulous beneath; secondaries slender, 5–7 per side, mostly flat on both surfaces, often obscure above, arcuate-ascending, indistinctly joined at the margins beneath; veinlet reticulation fine, mostly indiscernible above; inflorescence axillary and terminal, racemiform, 3–17 cm. long, many-flowered, the axillary ones confined to a pair in the uppermost axils; flowers not secund, more or less erect; peduncles slender, dark, 0.8–2.8 cm. long, glabrous; rachis slender, dark, glabrous or very slightly puberulent-pilose with obscure and scattered minute hairs; pedicels slender, 1–2 mm. long, very densely strigose-puberulent with incanous hairs, elongate to 6 mm. in fruit, usually erect; bractlets stipitate, linear or oblong, 3–9 mm. long, rather conspicuous, minutely pilose, long-persistent; calyx campanulate, about 2.5 mm. long, rather densely appressed-strigillose or puberulent with incanous hair, 1.5–2 mm. wide, indistinctly costate, its rim truncate, very minutely and obscurely 5-apiculate; corolla hypocrateriform, its tube 4.5–6 mm. long, rather narrow-cylindric, straight or curved, the lower portion glabrous outside, the upper portion densely appressed pulverulent-puberulent, its limb 5-parted, about 5 mm. in diameter, densely pulverulent-puberulent on both surfaces; fruiting-calyx enlarged, leathery, nigrescent in drying, obscurely appressed-puberulent or glabrescent, obvolute, including the (immature?) fruit, not beaked.

COLOMBIA—TERRITORIO DEL CAQUETÁ: Rio Caquetá, Tres Esquinas, *Georg N. Woronow & S. Juzepczuk 6243*, July 22, 1926, TYPE; in the herbarium of the Field Museum of Natural History at Chicago. Named in honor of G. N. Woronow.

Ghinia Cardenasi Moldenke, Geogr. Distrib. 28, nom. nud. (1939), sp. nov. Herba sublignosa; ramis acute tetragonis costatis densissime puberulis; nodis annulatis; petiolis densissime puberulis; laminis chartaceis ovatis vel rare ellipticis argute serratis, ad basim abrupte acutis vel subtruncatis, utrinque plusminus puberulis vel supra subglabratis; inflorescentiis axillaribus spicatis; pedunculis rhachideque densiuscule incano-puberulis tetragonis costatis.

Herb, about 50 cm. tall, woody at base; branches slender, acutely tetragonal, very densely puberulent throughout, longitudinally ribbed; nodes annulate, not ampliate; principal internodes 1–3.5 cm. long; leaves decussate-opposite; petioles slender, 3–6 mm. long, very densely puberulent like the branchlets; blades chartaceous, rather uniformly green on both surfaces or somewhat lighter beneath, ovate or rarely elliptic, 1.5–3.5 cm. long, 0.8–2 cm. wide, obtuse (in outline) at apex, sharply serrate from almost the base to the apex with acute antrorse teeth, abruptly acute or subtruncate at base, densely puberulent above and densely canescent beneath when young, very obscurely puberulent or subglabrate above in age, more plainly puberulent beneath; midrib slender, subimpressed above, prominulous beneath; secondaries slen-

der, 6–8 or more per side, close together, subparallel, straight and ascending, mostly extending direct to the sinuses between the teeth and secondarily into the teeth themselves, mostly subimpressed above, prominulous beneath; veinlet reticulation sparse, obscure on both surfaces; inflorescence axillary, spicate, 4.5–18 cm. long, many-flowered, the flowers during anthesis barely overlapping, the lowermost separate; peduncles (3–5 cm. long) and rachis slender, rather densely incanous-puberulent throughout, tetragonal and costate; pedicels obsolete; bractlets linear or filiform, 3–4 mm. long, puberulent; calyx tubular, 4–5 mm. long, 1–1.5 mm. wide, 5-costate, hyaline between the ribs, densely incanous-puberulent, its rim 5-apiculate, the apiculations filiform and 1–1.5 mm. long; corolla hypocrateriform, lilac, its tube cylindric, curved, 5–7 mm. long, its limb 5–7 mm. in diameter; fruiting-calyx spreading-campanulate, about 6 mm. long and to 6 mm. in diameter, appressed-puberulent, its rim scalloped and long-apiculate, the apiculations filiform and about 2 mm. long; fruit obovate, the body about 6 mm. long and 5 mm. wide, glabrous, prominently reticulate at apex, with 3 divergent horns to 4 mm. long, sharp-pointed and spine-like.

BOLIVIA—SANTA CRUZ: Ipias—Chiquitos, in grassy pampas, alt. 230 m. *Martin Cárdenas 2946*, in October, 1934, TYPE; in the herbarium of the Field Museum of Natural History at Chicago.

Lantana boyacana Moldenke, sp. nov. Frutex; ramis ramulisque densiuscule puberulis et patenti-pilosis glabrescentibus; nodis annulatis; foliis oppositis; petiolis plusminus marginatis dense adpresso-puberulis et patenti-pilosis; laminis chartaceis lanceolatis vel ovatis, ad apicem acutis vel subacutis, regulariter crenato-serrulatis utrinque dense velutino-puberulentibus et parce longeque pilosis, supra plerumque subbullatis; bracteolis magnis foliaceis ellipticis vel ovatis vel obovatis.

Shrub; branches and branchlets slender, obscurely tetragonal or subterete, rather more plainly tetragonal when young, rather densely puberulent and also spreading-pilose with cinereous hair, glabrescent in age; nodes annulate; principal internodes 0.6–5.8 cm. long; leaves decussate-opposite; petioles slender, 4–10 mm. long, more or less margined (especially toward the apex), densely appressed-puberulent and spreading-pilose with cinereous hair, the long pilose hairs abundant and 2 or 3 times as long as the puberulent ones; blades chartaceous, gray-green on both surfaces, lighter beneath, lanceolate or ovate, 1.7–5 cm. long, 1.1–3.5 cm. wide, acute or subacute at apex, regularly crenate-serrulate along the whole margins except at the very base with rounded, appressed, minute teeth, densely velutinous-puberulent with grayish or sordid hair on both surfaces, interspersed with much longer pilose hairs on the larger venation, often subbullate above; midrib slender, flat above, prominulous beneath; secondaries slender, 5–8 per side, arcuate-ascending, rather straight and not much arcuate, flat or subimpressed above, prominulous beneath; vein and veinlet reticulation abundant, mostly more or less impressed above, flat or prominulous beneath; inflorescence axillary, capitate; peduncles slender, 2.5–6.5 cm. long, rather densely incanous-puberulent and long-pilose like the branchlets; heads hemispheric, 1.5–2.5 cm. wide, densely many-flowered; involucral bractlets large and conspicuous, foliaceous, elliptic, ovate, or obovate, the lowermost ones 5–11 mm. long, 2.5–7.5 mm. wide, sessile, acute or abruptly short-acuminate at apex, densely short-pubescent on both surfaces with sub-

strigose incanous hair; receptacle to 6 mm. long; corolla about 7 mm. long, its limb about 4 mm. wide.

COLOMBIA—BOYACÁ: Valle de la Uvita, woods and thickets near Uvita, alt. 2490–2560 m., *J. Cuatrecasas 1851*, September 16, 1938, TYPE; in the herbarium of the Instituto Botanico, Bogotá, Colombia.

Lantana soatensis Moldenke, sp. nov. Frutex; ramis ramulisque gracillimis dense albido-strigosis subglabrescentibus; nodis annulatis; foliis oppositis numerosis saepe fasciculatis; petiolis dense albido-strigosis; foliis chartaceis ellipticis vel ovatis, ad apicem acutis, ad basim acuminatis, argute serrulatis utrinque dense sericeo-strigosis; bracteolis ovatis.

Shrub; branches and branchlets very slender, gray, obtusely tetragonal, densely strigose with short and appressed white hair, less densely so or even glabrescent in age; nodes annulate on young branchlets, less plainly so on older ones; principal internodes 0.5–6 cm. long; leaves decussate-opposite, abundant, often bearing additional fascicles on much abbreviated twigs in their axils; petioles slender, 3–6 mm. long, densely strigose with white hair like the branchlets; blades chartaceous, elliptic or ovate, 0.6–2.5 cm. long, 0.4–1.6 cm. wide, acute at apex, sharply serrulate from the widest part to the apex, acuminate at base, densely sericeous-strigose on both surfaces with white antrorse closely appressed hair, more densely so on the larger venation beneath; midrib slender, subimpressed above, prominulous beneath; secondaries very slender, 4–6 per side, arcuate-ascending, subimpressed above, prominulous beneath; veinlet reticulation obscure or indiscernible on both surfaces; inflorescence axillary, capitate; peduncles very slender, 1–2.5 cm. long, densely white-strigose like the branchlets; heads ovate-elongate, to 1 cm. wide and 1.5 cm. long, densely many-flowered; involucral bractlets ovate, sessile, the lowermost to 6 mm. long and 3 mm. wide, sharply acute at apex, densely white-strigose on both surfaces; corolla about 5 mm. long.

COLOMBIA—BOYACÁ: Soatá, arid slopes, alt. 2130 m., *J. Cuatrecasas 1031*, September 6, 1938, TYPE; in the United States National Hebarium at Washington.

THE NEW YORK BOTANICAL GARDEN,
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INDEX TO AMERICAN BOTANICAL LITERATURE

The aim of this Index is to include all current botanical literature written by Americans, published in America, or based upon American material; the word America being used in the broadest sense.

Papers that relate exclusively to bacteriology, forestry, agriculture, horticulture, manufactured products of vegetable origin, or laboratory methods are not included. If users of the Index will call the attention of the editor to errors or omissions, their kindness will be appreciated.

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